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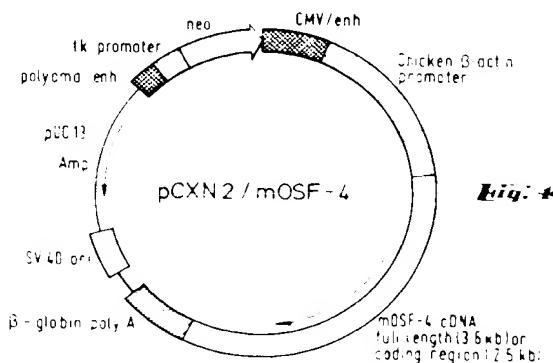
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Bone-related cadherin-like protein and process for its production.

A bone-related protein named OSF-4 which is obtained from bone tissue of a mammal including mouse or human, and a process for its production. This protein is a novel naturally occurring mammal protein of the cadherin family.

OSF-4 acts as an adhesion molecule or a growth factor which takes part in the process of osteogenesis at the site of bone induction. OSF-4 can be used as an agent for treating bone metabolic diseases, and its high organ specificity for bones enables its use as a diagnostic reagent for bone metabolic diseases.



This invention provides a novel bone-related protein. It is named OSF-4 and belongs to a group of cadherin molecules. The OSF-4 can be obtained from bone tissue of a mammal including mouse or human. This bone-related protein is useful for the diagnosis and treatment of bone metabolic diseases.

Bone metabolic diseases include osteoporosis, Paget's disease, osteomalacia, hyperostosis, and osteopetrosis. Osteoporosis, in particular, has a high incidence enough to affect about more than a half of postmenopausal women and elderly people, and effective methods for its diagnosis and treatment have been strongly desired.

Bone metabolic diseases involve some disorder of bone metabolism at the cellular level in bone tissue. The discovery, isolation and identification of factors associated specifically with bone metabolism are very effective for elucidating this disorder.

A cell line of osteoblasts which play a major role in osteogenesis, has been used and a proteinaceous factor produced specifically by this cell line has been identified. Therefore, the present invention provides a novel protein named OSF-4 which is substantially bone-specific, and which is highly homologous with various known cadherin type cell adhesion molecules in terms of amino acid sequence.

The OSF-4 can also be produced from the DNA sequence described in the present specification by an ordinary genetic engineering technique known in the art. Furthermore, the OSF-4 or its fragment can be produced from the amino acid sequence described in the specification by a chemical peptide synthesis method. Moreover, that fragment of the DNA sequence of the OSF-4 described in the present invention which is specifically different from other cadherin molecules can be synthesized with a length of 15 to 50 bases by an ordinary chemical oligonucleotide synthesis method. That fragmentary sequence can be used as a DNA probe for finding and identifying bone-derived cells. This identification of bone-derived cells is useful particularly for grasping the origin of metastatic or recurrent carcinoma, thus leading to an appropriate therapy for recurrent cancer. Of the partial peptides of the OSF-4, the peptide in the epitope portion that can be recognized by antibodies is usable for preparing a monoclonal antibody specific for the OSF-4. The resulting monoclonal antibody is of marked value for identifying bone-derived cells by an immunological cell tissue staining method. Because of its similarity to cell adhesion molecules, the OSF-4 is also useful for the treatment of fracture.

OSF-4 is a bone-specific cadherin-like proteinaceous factor. The following is known about cadherin which is a cell adhesion molecule involved in morphogenesis

The segmentation of cell population is one of the most basic elements for the construction of an animal body. This segmentation begins at a very early stage of morphogenesis. As the differentiation of cells proceeds, the same types of cells migrate and become reorganized in an orderly manner, thereby performing morphogenesis as well as the construction and maintenance of tissues. One of the elements that control such cellular migration is the selective adhesion of cells. Cells have the features of recognizing adjacent cells or adjacent extracellular matrices, and adhering to only particular ones. In accordance with the differentiation of cells, their adhesion specificities vary. Consequently, these cells may leave particular sites, migrating to and gathering at the sites where they should have originally been situated. So far, numerous cell adhesion molecules have been identified and all show cell type specificities. These molecules can be roughly classified into more than 4 groups, i.e. cadherin family, immunoglobulin superfamily adhesion molecules, integrin superfamily, selectins, and those not belonging to these categories (Hynes et al., (1992) Cell, vol. 68, pp. 303-322)

Cadherins, in particular, have been well analyzed, and not only their structures, but their functions have also been extensively studied. Cadherins are glycoproteins with molecular weights of about 120 kD, and are Ca^{2+} -dependent intercellular adhesion molecules (Takeichi, (1991), Science, vol. 251, pp. 1451-1455). Nearly 10 types of them have been identified. The respective types have binding specificities, and the same molecules react homophilically with each other. As a result, cells having the same type of cadherin bind selectively to each other. Thus, cadherins are considered indispensable for determining the specificities of intercellular adhesion. Typical examples are E-cadherin (epithelial cadherin), P-cadherin (placental cadherin), N-cadherin (neural cadherin), and L-CAM (liver cell adhesion molecule). All of them are similar in structure; each is composed of an extracellular region comprising 4 to 5 repeats of about 110 amino acids, a transmembrane region, and a cytoplasmic region comprising about 150 amino acids. Comparisons among the respective subclasses have shown about 50% identity of the amino acids. In the extracellular region, the identity rate is higher at a site nearer the N-terminus; it becomes gradually lower the nearer the transmembrane region; and it is maximal in the cytoplasmic region. In recent years, new cadherins have been reported, including, for instance, M-cadherin (muscle cadherin), B-cadherin (brain cadherin), T-cadherin (truncated cadherin), and desmoglein localized in desmosomes. These cadherins may originate from a single ancestor gene, and constitute the cadherin family.

5 Cleic studies of a cadherin molecule have shown that sites binding to calcium ions and sites determining binding specificity are present in its N-terminal region. The cytoplasmic domain has been found to be functionally important for the adhesion property, and to bind to a protein such as catenin or actin. Through these functions, cadherins have been suggested to contribute to cytoplasmic signaling. During 10 invasion and metastasis of cancer cells, these cadherins undergo quantitative changes, and so their relationship with oncogenesis has attracted a broad attention. Despite many such reports of cadherin molecules, there have been no reports of cadherin molecules with specificity for osteoblasts.

15 Bone formation and maintenance are dependent on the balance between osteoblasts which form bones and osteoclasts which resorb bones. Osteoblasts are mesenchymal cells of the same origin as myoblasts and adipocytes while osteoclasts originate from stem cells as do neutrophils and macrophages. Osteoclasts are known to express vitronectin receptors which belong to the integrin family. With osteoblasts the presence of cell adhesion molecules has only been suggested.

20 The object of the present invention is to find a new type of cell adhesion factor which is specifically expressed in osteoblasts. Such a new cell adhesion factor is an important molecule for the proliferation, differentiation, migration and reorganization of osteoblasts. This substance can be expected to find use in the diagnosis and treatment of various bone metabolic diseases.

25 cDNA of mouse OSF-4 (mOSF-4) was isolated from a mouse osteoblastic cell line MC3T3-E1 cDNA library constructed by a combination of PCR (polymerase chain reaction) and the subtraction method, and by differential screening. Then, the mouse OSF-4 cDNA was used as a probe for screening cDNA libraries obtained from human osteosarcoma cells. As a result, two types of OSF-4, named hOSF-4-1 and hOSF-4-2, 30 were obtained, and their nucleotide sequences were determined. The nucleotide sequence of the OSF-4 is very well conserved between mouse and human. Comparisons between mOSF-4 and hOSF-4-1 as well as hOSF-4-2 show 97.1% and 96.4% identity in amino acid level, respectively (Tables 1 to 3). The very high conservation between these species suggests that OSF-4 has essential roles in vertebrate bone metabolism. OSF-4 can be isolated and purified from the bone extracts of other vertebrates. The OSF-4 of other animal species can be obtained from cDNA libraries or genomic libraries constructed from their bones, cultured bone cells and other body tissues by recombinant gene technology using the cDNA of the present invention or its DNA fragment as a probe. Search through the currently available DNA and amino acid sequence data bases demonstrated the sequence of the cDNA in the present invention to be novel.

35 In comparison with the amino acid sequence of mOSF-4, hOSF-4-1 shows high conservation in the whole domain. hOSF-4-2, on the other hand, is completely identical with hOSF-4-1 in terms of the N-terminal to 631st amino acid residues. Because of the insertion of 179 bases into the transmembrane region, however, frameshift occurs, with the result that the 62 amino acid residues ranging from the 632nd amino acid residue to the 693rd-position C-terminus assume a completely different structure (Tables 1 to 3). Hence, the C-terminal 9 amino acid residues in the transmembrane region, and the cytoplasmic region 40 are completely different between hOSF-4-1 and hOSF-4-2. Such cadherin with the C-terminal region deleted corresponds to T-cadherin. This type of cadherin has been suggested to take part in the control of cell adhesion.

45 A peptide corresponding to 15 hydrophilic amino acid residues at the 101st to 115th positions in the EC1 domain of mOSF-4 was chemically synthesized. This peptide was conjugated with KLH (keyhole limpet hemocyanin), and used for immunization of rabbits. The resulting anti-mOSF-4 peptide antisera were used for immunohistochemical detection of OSF-4 in systemic slices of the neonatal mouse. OSF-4 was detected in the osteoblasts, chondrocytes and so on.

50 Generally, the OSF-4 can be directly extracted from bone tissue or cartilage tissue of a human, bovine, murine or other source by a known biochemical technique. The DNA coding for the OSF-4 can be obtained by constructing a cDNA library or a genomic library from mRNA extracted from vertebrate bone tissue, and using a probe comprising a labeled fragment of the mouse DNA sequence disclosed in the present specification. A full length cDNA clone can be obtained by a combination of the above-described and other standard techniques on molecular biology.

55 As described above, OSF-4 shows homology with known representative cadherin molecules, but it is a cadherin molecule belonging to a new subclass different from those so far reported. Its structure is composed of 5 repeats in an extracellular region, a transmembrane region, and a cytoplasmic region (Fig. 1). Comparisons between OSF-4 and other cadherins have shown that homology in the extracellular region becomes lower from the N- terminus toward the transmembrane region and the highest homology is noted in the cytoplasmic region, according to the homology pattern among the existing different cadherin molecules (Table 4).

Table 4

Comparisons of amino acids among mouse OSF-4 and other cadherin molecules								
Types of cadherin compared	Homology (%)							
	EC1	EC2	EC3	EC4	EC5	TM	CP	MP
OSF-4:N	39.3	46.8	32.8	29.8	27.4	59.4	50.3	39.4
	32.7	40.4	30.7	30.8	17.2	34.4	47.1	33.9
	34.6	35.8	36.0	29.1	19.0	23.1	43.8	33.3
	31.8	38.5	27.6	26.0	25.4	34.4	45.1	33.2
	60.2	53.5	45.1	43.9	27.0	46.9	64.1	49.8
	51.9	51.8	45.1	49.5	27.9	30.8	57.7	47.3
	65.7	61.1	52.7	46.7	38.9	53.8	79.7	58.7

In Table 4, the homology of amino acids in each region was calculated and expressed in %. The abbreviations are as follows: EC1 to EC5, five extracellular regions; TM, transmembrane region; CP, cytoplasmic region; MP, mature protein; N, N-cadherin; E, E-cadherin; P, P-cadherin; and M, M-cadherin. In the column "Types of cadherin compared," OSF-4:N denotes comparisons of the amino acid sequences in the respective regions between OSF-4 and N-cadherin (the same is true for the other combinations).

The protein provided by the present invention is a group of glycoproteins, named OSF-4, which belongs to a new cadherin subclass and plays an important role in osteogenesis. More concretely, the human and mouse OSF-4 proteins described in this specification are included. OSF-4 is expressed in osteoblasts during the process of bone formation, and acts as a cell adhesion molecule and a morphogenesis-related substance. These human and mouse OSF-4 proteins can be used to identify and isolate other mammalian OSF-4 proteins similar in DNA sequence and amino acid sequence.

The present invention further provides polypeptides comprising analogues of OSF-4, i.e. mutants and fused proteins having OSF-5 activity, as well as fragments of the OSF-4 which can be identified as OSF-4 related, particularly with at least 10, preferably 15 amino acids. The cDNA of mouse OSF-4 isolated from the mouse osteoblastic cell line MC3T3-E1 encodes a protein consisting of 796 amino acids, including a signal peptide composed of 24 amino acid residues. There are two isoforms of human OSF-4 which were isolated from a human osteosarcoma cDNA library. One cDNA clone, human OSF-4-1, encodes a protein consisting of 796 amino acids including a signal peptide composed of 24 amino acid residues. The other cDNA clone, human OSF-4-2, encodes a protein consisting of 693 amino acids including a signal peptide composed of 24 amino acid residues. The present invention also provides a process for producing OSF-4 by recombinant DNA technology.

According to the present application the term "hybridization under stringent conditions" means hybridization conditions with a salt concentration of 6 x SSC (NaCl-citrate buffer) at 62-68 °C.

40 Brief Explanation of Tables and Figures

Table 1 shows an alignment of the amino acid sequences of mouse OSF-4, human OSF-4-1 and human OSF-4-2. Common amino acid residues are shown in the form of consensus.

Table 2 shows a continuation of an alignment of the amino acid sequences of mouse OSF-4, human OSF-4-1 and human OSF-4-2 shown in Table 1. Common amino acid residues are shown in the form of consensus.

Table 3 shows a continuation of an alignment of the amino acid sequences of mouse OSF-4, human OSF-4-1 and human OSF-4-2 shown in Table 2. Common amino acid residues are shown in the form of consensus.

Figure 1 is a schematic drawing of the structure of mouse OSF-4 precursor protein. OSF-4 precursor protein is divided into eight regions, a signal region (shaded part), five extracellular regions (EC1, EC2, EC3, EC4 and EC5), a transmembrane region (TM) and a cytoplasmic region (CP).

Figure 2 shows a restriction enzyme map of cDNA coding for mouse OSF-4. The bold letters indicate the region coding for the amino acid of OSF-4. There are no KpnI and Sall sites in the map.

Figure 3 shows the tissue-specific expression of mouse OSF-4. This was analyzed by purifying RNA from various tissue and cultured cells followed by RNA dot blotting. This diagram shows the results of autoradiography.

Figure 4 shows the map of expression vector pMSS60. It is mentioned that the content of the Japanese

Priority application NC 230028192 is also part of the present application

Examples

5 The present invention will be described in more detail by reference to the following Examples.

Example 1

Construction of cDNA library by subtraction and PCR

10 The construction of a cDNA library specific for the osteoblastic cell line MC3T3-E1 will be hereinafter described. This cDNA library is constructed from MC3T3-E1 cDNA library by a combination of the subtraction method and the PCR with the gene expressed in mouse liver tissue being subtracted. Each cDNA clone has gene fragments with an average length of about 300 bases, and is characterized in that the 15 gene with a low content has been amplified too.

Unless otherwise specified, all general recombinant DNA protocols complied with Sambrook et al., "Molecular Cloning Manual" (1989), Cold Spring Harbor Laboratory, Cold Spring Harbor, U.S.A. Total RNAs were extracted from 8×10^7 MC3T3-E1 cells and about 1 g of mouse liver tissue by the guanidine method. Poly A⁺RNAs were purified from the total RNAs by means of the commercially available product "Oligo dT 20 Latex mRNA Purification Kit" (Takara Shuzo). cDNAs were synthesized by a cDNA synthesis kit (Amersham) using 1 μ g of each poly A⁺RNA as a template. However, a random primer was used, instead of an oligo dT primer, in an amount of 1.5 times its ordinary amount used, whereby the cDNA chain elongation was restricted to an average length of about 300 bases. After the cDNAs were made double-stranded and blunt-ended by use of the above kit, they were joined with T4DNA ligase (Takara Shuzo) to the following 25 two DNA linkers, i.e. ATOS-1.2 (Sequence ID Nos. 4 and 5 of the Sequence Listing) for the MC3T3-E1 cDNA, and ATOS-4.5 (Sequence ID Nos. 6 and 7 of the Sequence Listing) for the liver cDNA:

ATOS-1/2:

30 ATOS-1 5'- CTCTTGCTTGAATTCTGGACTA-3'
ATOS-2 3'-ACACGAGAACGAACCTAAGCCTGAT-5'

ATOS-4/5:

35 ATOS-4 5'- CTCTTGCTTAAGCTTGGACTA-3'
ATOS-5 3'-ACACGAGAACGAATTCTGAACCTGAT-5'

40 Then, each reaction product was subjected to DNA amplification by the PCR (polymerase chain reaction) method using ATOS-1 and ATOS-4, respectively, as primers. The amplified DNA concentration was determined with the DNA assay kit "DNA Dipstick" (Invitrogen). The subtraction method was performed using photobiotin (Pierce). Photobiotin (20 ng) was added to 20 μ g of the PCR-amplified liver cDNA, and light from a sunlamp 10 cm apart was projected onto the liver cDNA for 10 minutes to label it with biotin. To 45 3.0 μ g of the labeled liver cDNA was added 0.3 μ g of unlabeled MC3T3-E1 cDNA for hybridization. Then, streptavidin (Takara Shuzo) was reacted, and the reaction mixture was extracted with phenol to remove cDNA common to the liver cDNA from the MC3T3-E1 cDNA. The subtraction method was repeated to remove as much of the common cDNA as possible from the MC3T3-E1 cDNA. DNA was amplified by PCR using the aforementioned ATOS-1, and the DNA concentration was measured. This cDNA (10 ng) was 50 digested with the restriction enzyme EcoRI, and then ligated with T4 ligase to 1 μ g of the phage vector lambda gt10 (lambda gt10 EcoRI cloning kit, Stratagene) which was digested with EcoRI and de-phosphorylated at its ends. The resulting recombinant DNA was packaged into lambda phage particles by use of the in vitro packaging kit "Gigapack-gold" (Stratagene). The recombinant phages were infected into E. coli C600 (preserved as HT003 at Japanese Cancer Research Resources Bank, National Institute of 55 Health of Japan), and the organisms were applied to an agar medium along with a soft agar medium to form phage plaques. The efficiency of infection was determined to be 3×10^6 phage plaques μ g vector DNA.

The resulting cDNA library was subjected to differential screening to select clones with a high specificity for MC3T3-E1. Concretely, 2.25×10^4 phages were applied to total 10 plates, and the resulting

plaques on each plate were transferred to two nylon membrane filters (total 20 filters). These series of plaques were subjected to plaque hybridization with radiolabeled MC3T3-E1 cDNA as the probe for one of the series, and with radiolabeled liver cDNA for the other series. In 273 clones, expression was observed with the MC3T3-E1 cDNA probe, but not with the liver cDNA probe. These clones were used as a mini-
5 library in subsequent experiments

Example 2

Isolation of mouse OSF-4 clone

10 A description will be made of methods to identify a cDNA fragment of OSF-4 as an MC3T3-E1 specific clone from the mini-library constructed in Example 1, and to clone full length cDNA from the cDNA library of MC3T3-E1 with the use of this fragment.

15 The total RNAs from MC3T3-E1 and liver prepared in Example 1 were spotted in an amount of 1 μ g each onto nylon membrane filters. 273 of the filters were prepared, and used for hybridization to be described later on. Separately, the DNA of the inserts of the 273 phage clones prepared in Example 1 was amplified by PCR. This DNA was agarose gel electrophoresed, and main bands were cut out, purified, and radiolabeled for use as a probe. A clone showing expression with MC3T3-E1 cDNA but no expression with liver cDNA upon autoradiography was recloned into a plasmid vector. Concretely, the DNA of the inserts 20 amplified by PCR and then purified was digested with the restriction enzyme EcoRI, and recloned into the EcoRI site of the plasmid vector pUC118 (Takara Shuzo). The DNA sequence of the resulting clone was determined with commercially available "DNA Sequence Kit" (Takara Shuzo) using a universal primer. Search through DNA and protein data bases showed that DNA sequence to constitute a clone homologous 25 with the existing cadherin. This clone was designated as D45, and used for subsequent cloning of the full length cDNA.

For cloning of the full length cDNA, blunt-ended double-stranded cDNA was synthesized with the cDNA synthesis kit "cDNA Synthesis System Plus" (Amersham) using 5 μ g of the poly A⁺RNA of MC3T3-E1 purified in Example 1. The resulting cDNA was ligated to EcoRI/NotI adaptor (Takara Shuzo) using T4 30 ligase, and the product was agarose gel electrophoresed to purify a fragment more than about 700 base pair long. This fragment was joined to the EcoRI site of lambda gt10 phage vector (Stratagene), and packaged into phage particles in the same way as in Example 1. The packages were infected into E. coli as in Example 1, and the efficiency of infection was determined to be 1.5×10^7 phage plaques/ μ g vector DNA. The aforementioned D45 was radiolabeled for use as a probe, and 1.0×10^6 phage clones of the cDNA 35 library were screened by plaque hybridization. Fourteen positive hybridization signals were obtained, whereafter the NotI fragment of the phage clone with the longest insert was recloned into the NotI site of the plasmid vector pGEM11Zf(+) (Stratagene). The resulting clone was designated as pKOT164.

Example 3

40 Determination of mouse OSF-4 DNA sequence

Deletion mutants of the pKOT164 and a subclone containing its cDNA fragment were prepared with "the Deletion Kit for Kilo Sequence" (Takara Shuzo) by cutting at intervals of about 300 base pairs in each 45 opposite direction. The DNA sequence of each deletion mutant was determined with the automatic DNA sequencer 373A (Applied Biosystems, U.S.A.). The entire DNA sequence of the cDNA, and an amino acid sequence translated from this DNA sequence are shown as Sequence ID No. 1 of the Sequence Listing. The protein encoded by this cDNA was designated as OSF-4. No. 1 of the amino acid residue corresponds to the N-terminus of the predicted OSF-4 precursor protein. The restriction enzyme map of that cDNA is shown in Fig. 2.

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Example 4

Tissue specific expression of mouse OSF-4

55 RNA dot blotting was performed to investigate the tissue specific expression of mouse OSF-4. The total RNAs of the thymus, spleen, brain, kidney, liver, lung, testis and heart of mice (purchased from Nippon Clea) were prepared by the guanidine method. Calvarial osteoblast-rich cells were obtained from a culture of newborn mice calvaria. Total RNA was extracted from these cells in the same way as described above.

One μ g of the total RNA each from the above-mentioned tissues, cultured calvarial cells, MC3T3-E1 and mouse fibroblast cell line NIH3T3 (ATCC CRL 1658) was dotted onto nylon membrane filters (Biodyne PALL) fixed by baking, and used for hybridization. Separately, the pKOT164 was digested with NotI, and isolated and purified by agarose gel electrophoresis. Then, the isolate was radiolabeled and used as a probe. Autoradiography indicated high expression for the cultured calvarial cells and MC3T3-E1, and low expression for the lung and testis (Fig. 3).

Example 5

10 Cloning of cDNA coding for human OSF-4

The NotI fragment containing the cDNA region of pKOT164 was purified and used as a probe to screen a human osteosarcoma cDNA library consisting of 1.3×10^6 clones. Twenty-one positive signals were obtained, and 5 clones were isolated. Two clones with large inserts were recloned into plasmid vector pHSG398. The resulting plasmids were designated as pKOT161 and pKOT170.

15 Example 6

Determination of human OSF-4 DNA sequence

20 From the pKOT161 and pKOT170 cloned in Example 5 and their subclones, deletion mutants were prepared in the same way as in Example 3. Then, their DNA sequences were determined. These DNA sequences and the amino acid sequences predicted from them are shown in Sequence ID Nos. 2 and 3 of the Sequence Listing. The proteins encoded by these cDNAs were designated as human OSF-4-1 and 25 human OSF-4-2. The amino acid residue No. 1 of each of them corresponds to the N-terminus of the predicted OSF-4 precursor protein.

Example 7

30 Preparation of anti-OSF-4 antisera

In preparing anti-peptide antibodies against mouse OSF-4, the corresponding 15 amino acid residues in the EC1 were synthesized by the solid phase synthesis method using a peptide synthesizer (430A, Applied Biosystems), in accordance with an experimental report on M-cadherin (Donalies et al., (1991), Proc. Natl. 35 Acad. Sci., U.S.A., vol. 88, pp. 8024-8028). The synthetic peptide was OSF-4, 1 (FVIDDKSGNIHATKT, Sequence ID No. 8 of the Sequence Listing). This synthetic peptide was conjugated with KLH (keyhole limpet hemacyanin) using glutaraldehyde as a coupling agent, and used for immunization of rabbits. The resulting antisera could be used to search immunohistochemically for the presence of OSF-4 in newborn mouse systemic slices, and to detect the expression of OSF-4 in *E. coli*, yeast and animal cells.

40 Example 8

Expression of OSF-4 in animal cells

45 The present example describes the preparation and expression of the expression vector for mouse OSF-4 in animal cells and the functional analysis of the produced OSF-4.

There is an open-reading frame in the 5'-flanking region of the OSF-4-coding region in the base sequence as shown in SEQ ID NO: 1 in Sequence Listing. The open-reading frame was expected to decrease the translation efficiency of OSF-4. Therefore, a clone which contained the OSF-4-coding region 50 alone was selected from the deletion mutants prepared in Example 3, and it was used for the preparation of the expression vector for OSF-4. The segment of the clone from G of the 191st to A of the 2700th in SEQ ID NO: 1 in Sequence Listing was cut and a linker containing Xhol and BamHI sites and a linker containing KpnI site were ligated to the 5'-terminus and 3'-terminus of the segment, respectively. Then the segment bordered with Xhol site was inserted into the Xhol site of an expression plasmid vector for animal cells, 55 pCXN2 (Niwa et al., (1991) Gene, vol. 108, p193-200). The OSF-4 expression vector obtained was termed pMSS60 (Fig. 4).

The pMSS60 was introduced into L-cells of a fibroblast cell line derived from mouse epidermis by the calcium-phosphoric acid co-precipitating method. Then 12 G418 resistant colonies transformed by pMSS60

were cultured separately to obtain the cloned cell lines. RNA was extracted from these cloned cells. Then, three OSF-4-high producer cloned cell lines were selected with an RNA dot blotting method by using mouse OSF-4 cDNA as the probe. The three clones were termed C1, C7 and C11, respectively.

5 A band of approximately 100 kDa reacting with anti-OSF-4 antibody was detected by Western blotting analysis out of the proteins produced in these cloned cells.

Furthermore, the functional analysis of OSF-4 produced by these cloned cell lines was conducted as follows by Takeuchi's aggregation assay method which had been originally established to examine cadherin cell adhesive properties (Takeichi et al., (1977) J. Cell Biol., vol. 75, p464-474).

10 First, from each monolayer cells (C1, C7 and C11), TC-and TE-treated cell suspensions were prepared.

The TC-treated cell suspensions were prepared as follows. The monolayer cells were rinsed three times with CMF solution (Puck's Ca^{2+} and Mg^{2+} free saline; J. Exp. Med. vol. 108, p954- 956, 1958) containing 1 mM calcium chloride. Then, the cell suspensions were incubated at 37 °C for 15 min with HCMF solution (HEPES-buffered saline; 8.0 g of NaCl, 0.4 g of KCl, 0.09 g of $\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$, 1 g of glucose, 2.38 g of HEPES, and 4.8 ml of 1N NaOH in 1.000 ml of H_2O , pH7.4) containing 0.01% trypsin and 0.1 mM calcium chloride.

15 The TE-treated cell suspensions were prepared by the same procedure as for preparation of TC-treated cell suspensions except incubation at 37 °C for 15 min with HCMF solution containing 1 mM EDTA and 0.01% trypsin.

20 Then, each cell suspension was rinsed with CMF solution twice and divided into cell suspensions containing 1×10^6 cells/3 ml in HCMF solution with or without 1 mM calcium chloride. Each cell suspension was transferred to a 15 ml-size conical tube and was allowed to make cell aggregation by stirring with 80 rpm at 37 °C for one hour. The ratio of N_1/N_0 was calculated by counting with a Coulter-counter the number of cells before stirring (N_0) and the number of cell clots after stirring (N_1). The results are shown in Table 2: the OSF-4- expressing cloned cell lines showed calcim-dependent cell adhesive property similar to cadherin. Cadherin molecule is known not to be digested by trypsin with calcium. Thus, the cell suspension treated with EDTA (TE-treated) were digested by trypsin and did not show any cell adhesion.

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[Table 3]

	601	650	700	750	796
HSF-4	LLSCNAEAYI	LNAGLSTGAL	IAILACIVIL	LVIVWLFVIL	RRQKKEPLIV
HSF-4-1	LLSCNAEAYI	LNAGLSTGAL	IAILACIVIL	LVIVWLFVIL	RRQKKEPLIV
HSF-4-2	LLSCNAEAYI	LNAGLSTGAL	IAILACIVIL	LVIVWLFVIL	RRQKKEPLIV
Consensus	LLSCNAEAYI	LNAGLSTGAL	IAILACIVIL	LVIVWLFVIL	RRQKKEPLIV
HSF-4	QMPRPGLRP	APNSVDDDF	INTRIGEADN	OPTAPPYOSI	QIYGEGRGS
HSF-4-1	QMPRPGLRP	APNSVDDDF	INTRIGEADN	OPTAPPYOSI	YLMWGRFRK
HSF-4-2	QLMLFSTVKV	WRRFCLLGIVF	IKLPFLYVA	TESPTLISL	KLADLYGSD
Consensus	Q-M	-	-	-	TFDDDS

SEQUENCE LISTING

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10
Title of Invention: Bone-related Cadherin-like Protein and Process for its Production
Number of Sequences: 8
Computer Readable Form:
Computer: 3,5" HD Diskette
Medium Type: 386 SX
Operating System: MS-DOS
Software: ASCII

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"Sequence Listing"

SEQ ID NO: 1
 SEQUENCE TYPE: nucleic acid
 SEQUENCE LENGTH: 3581 base pairs

STRANDEDNESS: double
 TOPOLOGY: linear
 MOLECULAR TYPE: cDNA to mRNA

ORIGINAL SOURCE
 ORGANISM: *Mus musculus*
 STRAIN: osteoblastic cell line MC3T3E1

FEATURE:
 SEQUENCE Description: SEQ ID NO: 1:

15	GAATTCCGGG CGGCCTTGA AGACATTCAG TTCTGTTATT TATTGAATGA CCAATCAGAT	60
	GGGTGGAGCA TGTTATAGGA ATTGGCAGCA GGTATCCAAT GGGTGAAGAA GAAGCTGACT	120
	GGGGAGGTGA CCAACCTGG CGTGATGTCC TCAGTGAGTG AAGATATTCC ATTCCAGAGG	180
	AGGTCTACTT GACACATCTG GGAGGCCGCC ATCCGAAAGA AAGCCACTCT GTTGGTGTAG	240
20	GGAGTGACAG CTGCATTCTC CTGTGCCCTAC TGCATAACCA AAA ATG AAG GAG AAC	295
	Met Lys Glu Asn	
	1	
25	TAC TGT TTA CAA GCT GCC CTG GTG TGC CTG AGC ATG CTA TAC CAC AGC	343
	Tyr Cys Leu Gln Ala Ala Leu Val Cys Leu Ser Met Leu Tyr His Ser	
	5 10 15 20	
	CAG GCG TTT GCT CTG GAG CGA CGA AGC CAC CTG CAT CCC TCT TTC CAT	391
30	Gln Ala Phe Ala Leu Glu Arg Arg Ser His Leu His Pro Ser Phe His	
	25 30 35	
	GGA CAC CAT GAG AAG GGC AAG GAG GGG CAG GTG CTG CAA CGC TCC AAG	439
	Gly His His Glu Lys Gly Lys Glu Gly Gln Val Leu Gln Arg Ser Lys	
35	40 45 50	
	AGA GGC TGG GTC TGG AAC CAA TTC TTT GTG ATA GAA GAG TAC ACC GGG	487
	Arg Gly Trp Val Trp Asn Gln Phe Phe Val Ile Glu Glu Tyr Thr Gly	
	55 60 65	
40	CCT GAC CCT GTG CTG GTG GGC AGG CTT CAT TCT GAC ATT GAC TCC GGT	535
	Pro Asp Pro Val Leu Val Gly Arg Leu His Ser Asp Ile Asp Ser Gly	
	70 75 80	
45	GAT GGG AAC ATT AAA TAC ATT CTC TCA GGT GAA GGA GCG GGA ACC ATT	583
	Asp Gly Asn Ile Lys Tyr Ile Leu Ser Gly Glu Gly Ala Gly Thr Ile	
	85 90 95 100	
	TTT GTG ATT GAT GAC AAA TCA GGG AAC ATT CAT GCC ACC AAG ACA TTG	631
50	Phe Val Ile Asp Asp Lys Ser Gly Asn Ile His Ala Thr Lys Thr Leu	
	105 110 115	

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	GAC CGA GAG GAG AGA GCC CAG TAC ACA CTG ATG GCT CAG GCG GTG GAC	679		
5	Asp Arg Glu Glu Arg Ala Gln Tyr Thr Leu Met Ala Gln Ala Val Asp			
	120	125	130	
	AGG GAC ACC AAC AGA CCA CTG GAG CCA CCT TCA GAA TTC ATT GTT AAG	727		
10	Arg Asp Thr Asn Arg Pro Leu Glu Pro Pro Ser Glu Phe Ile Val Lys			
	135	140	145	
	GTC CAG GAC ATT AAT GAC AAC CCT CCA GAG TTT CTG CAT GAA ATC TAT	775		
15	Val Gln Asp Ile Asn Asp Asn Pro Pro Glu Phe Leu His Glu Ile Tyr			
	150	155	160	
	CAT GCC AAT GTG CCT GAG AGG TCC AAT GTG GGA ACA TCA GTT ATC CAA	823		
20	His Ala Asn Val Pro Glu Arg Ser Asn Val Gly Thr Ser Val Ile Gln			
	165	170	175	180
	GTC ACA GCC TCT GAT GCA GAT GAT CCC ACC TAT GGA AAT AGT GCC AAG	871		
25	Val Thr Ala Ser Asp Ala Asp Asp Pro Thr Tyr Gly Asn Ser Ala Lys			
	185	190	195	
	TTA GTG TAT AGC ATC CTT GAA GGA CAA CCC TAT TTC TCG GTG GAG GCC	919		
30	Leu Val Tyr Ser Ile Leu Glu Gly Gln Pro Tyr Phe Ser Val Glu Ala			
	200	205	210	
	CAA ACA GGT ATC ATC AGG ACA GCC CTT CCC AAT ATG GAC AGA GAA GCC	967		
35	Gln Thr Gly Ile Ile Arg Thr Ala Leu Pro Asn Met ASP Arg Glu Ala			
	215	220	225	
	AAG GAG GAG TAC CAC GTG GTG ATC CAG GCC AAG GAC ATG GGT GGA CAC	1015		
40	Lys Glu Glu Tyr His Val Val Ile Gln Ala Lys Asp Met Gly Gly His			
	230	235	240	
	ATG GGT GGA CTC TCA GGG ACA ACC AAA GTG AGC ATC ACT CTG ACT GAT	1063		
45	Met Gly Gly Leu Ser Gly Thr Thr Lys Val Thr Ile Thr Leu Thr Asp			
	245	250	255	260
	GTC AAC GAC AAC CCA CCA AAG TTT CCA CAG AGC GTG TAC CAG ATG TCT	1111		
50	Val Asn Asp Asn Pro Pro Lys Phe Pro Gln Ser Val Tyr Gln Met Ser			
	265	270	275	
	GTA TCA GAA GCA GCT GTC CCG GGG GAG GAA GTA GGA AGG GTG AAG GCT	1159		
	Val Ser Glu Ala Ala Val Pro Gly Glu Glu Val Gly Arg Val Lys Ala			
	280	285	290	
	AAA GAC CCA GAC ATT GGA GAA AAT GGC TTA GTC ACA TAC AAT ATC GTT	1207		
	Lys Asp Pro Asp Ile Gly Glu Asn Gly Leu Val Thr Tyr Asn Ile Val			
	295	300	305	
	GAT GGA GAC GGC ATA GAA ATT GCA ATT ACA ACA GAC TAT GAA ACA	1255		
	Asp Gly Asp Gly Ile Glu Leu Phe Glu Ile Thr Thr Asp Tyr Glu Thr			
	310	315	320	

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	CAG GAT GGT GTG GTG AAG CTG AAA AAG CCT GTC GAT TTT GAA ACC AAA	1303		
	Gln Asp Gly Val Val Lys Leu Lys Lys Pro Val Asp Phe Glu Thr Lys			
325	330	335	340	
	AGA GCT TAT AGC TTG AAG ATA GAG GCC GCC AAT GTT CAC ATT GAT CCG	1351		
	Arg Ala Tyr Ser Leu Lys Ile Glu Ala Ala Asn Val His Ile Asp Pro			
	345	350	355	
	AAG TTC ATC AGC AAT GGA CCT TTC AAG GAC ACT GTG ACC GTC AAG ATT	1399		
	Lys Phe Ile Ser Asn Gly Pro Phe Lys Asp Thr Val Thr Val Lys Ile			
	360	365	370	
	TCA GTC GAA GAT GCC GAT GAG CCT CCC ATG TTC TTG GCC CCA AGT TAT	1447		
	Ser Val Glu Asp Ala Asp Glu Pro Pro Met Phe Leu Ala Pro Ser Tyr			
	375	380	385	
	ATC CAT GAA GTT CAA GAA AAT GCA GCT GCT GCC ACT GTG GTT GGG AGA	1495		
	Ile His Glu Val Gln Glu Asn Ala Ala Ala Gly Thr Val Val Gly Arg			
20	390	395	400	
	GTC CAT GCC AAA GAC CCA GAT GCT GCC AAC AGC CCA ATA AGG TAT TCA	1543		
	Val His Ala Lys Asp Pro Asp Ala Ala Asn Ser Pro Ile Arg Tyr Ser			
	405	410	415	420
25	ATT GAT CGT CAT ACT GAC CTC GAC AGG TTT TTC ACG ATT AAT CCA GAA	1591		
	Ile Asp Arg His Thr Asp Leu Asp Arg Phe Phe Thr Ile Asn Pro Glu			
	425	430	435	
	GAT GGT TTT ATT AAA ACT ACG AAA CCT CTA GAT AGG GAA GAA ACT GCC	1639		
30	Asp Gly Phe Ile Lys Thr Thr Lys Pro Leu Asp Arg Glu Glu Thr Ala			
	440	445	450	
	TGG CTC AAC ATC TCT GTC TTC GCA GCA GAA ATT CAC AAC AGA CAT CAG	1687		
	Trp Leu Asn Ile Ser Val Phe Ala Ala Glu Ile His Asn Arg His Gln			
35	455	460	465	
	GAA ACC AAA GTC CCA GTG GCC ATC AGG GTC CTG GAT GTC AAT GAC AAT	1735		
	Glu Thr Lys Val Pro Val Ala Ile Arg Val Leu Asp Val Asn Asp Asn			
	470	475	480	
40	GCT CCT AAG TTT GCT GCC CCT TAT GAA GGT TTT ATC TGT GAG AGC GAT	1783		
	Ala Pro Lys Phe Ala Ala Pro Tyr Glu Gly Phe Ile Cys Glu Ser Asp			
	485	490	495	500
	CAC CCC AAG GCA CTC TCC AAC CAG CCA ATA GTT ACA GTT AGT GCA GAT	1831		
45	His Pro Lys Ala Leu Ser Asn Gln Pro Ile Val Thr Val Ser Ala Asp			
	505	510	515	
	GAC CAG GAC GAC ACA GCA AAT GGA CCA AGA TTT ATC TTC AGC CTA CCC	1879		
	Asp Gln Asp Asp Thr Ala Asn Gly Pro Arg Phe Ser Leu Pro			
50	520	525	530	

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	CCT GAA ATC ATG CAC AAC CCA AAC TTC ACA GCA AGA GAC AAC AGA GAT	1927
	Pro Glu Ile Met His Asn Pro Asn Phe Thr Val Arg Asp Asn Arg Asp	
5	535 540 545	
	AAC ACT GCA GGA GTA TAT GCC CGA CGT GGA GGG TTC AGT CGG CAG AAG	1975
	Asn Thr Ala Gly Val Tyr Ala Arg Arg Gly Gly Phe Ser Arg Gln Lys	
	550 555 560	
10	CAG GAC TTC TAC CTC CTG CCC ATT GTG ATC AGT GAT GGT GGC ATT CCA	2023
	Gln Asp Phe Tyr Leu Leu Pro Ile Val Ile Ser Asp Gly Gly Ile Pro	
	565 570 575 580	
	CCT ATG AGT AGC ACC AAT ACC CTC ACT ATC AAA GTC TGT GGC TGT GAT	2071
	Pro Met Ser Ser Thr Asn Thr Leu Thr Ile Lys Val Cys Gly Cys Asp	
15	585 590 595	
	GTG AAT GGG GCA CTG TTG TCC TGT AAC GCT GAA GCC TAC ATC CTG AAT	2119
	Val Asn Gly Ala Leu Leu Ser Cys Asn Ala Glu Ala Tyr Ile Leu Asn	
	600 605 610	
20	GCC GGT CTG AGC ACT GGG GCA CTG ATC GCC ATC CTT GCC TGC ATC GTC	2167
	Ala Gly Leu Ser Thr Gly Ala Leu Ile Ala Ile Leu Ala Cys Ile Val	
	615 620 625	
25	ATT CTT CTG GTC ATC GTT GTG CTG TTT GTT ACC CTG AGG AGG CAA AAG	2215
	Ile Leu Leu Val Ile Val Val Leu Phe Val Thr Leu Arg Arg Gln Lys	
	630 635 640	
	AAA GAA CCA CTC ATT GTC TTT GAA GAG GAG GAT GTC CGT GAG AAC ATC	2263
	Lys Glu Pro Leu Ile Val Phe Glu Glu Glu Asp Val Arg Glu Asn Ile	
30	645 650 655 660	
	ATA ACC TAT GAT GAT GAA GGG GGT GAG GAA GAC ACT GAA GCC TTC	2311
	Ile Thr Tyr Asp Asp Glu Gly Gly Glu Glu Asp Thr Glu Ala Phe	
	665 670 675	
35	GAC ATA GCC ACC CTG CAG AAT CCT GAC GGC ATC AAT GGA TTT ATC CCT	2359
	Asp Ile Ala Thr Leu Gln Asn Pro Asp Gly Ile Asn Gly Phe Ile Pro	
	680 685 690	
40	CGC AAA GAT ATC AAA CCT GAG TAT CAG TAT ATG CCT AGA CCT GGG CTG	2407
	Arg Lys Asp Ile Lys Pro Glu Tyr Gln Tyr Met Pro Arg Pro Gly Leu	
	695 700 705	
	CGA CCA GCA CCC AAC AGT GTG GAT GTG GAC GAC TTC ATC AAC ACA AGA	2455
45	Arg Pro Ala Pro Asn Ser Val Asp Val Asp Asp Phe Ile Asn Thr Arg	
	710 715 720	
	ATA CAG GAG GCA GAT AAT GAT CCC ACA GCC CCT CCC TAT GAC TCC ATC	2503
	Ile Gln Glu Ala Asp Asn Asp Pro Thr Ala Pro Pro Tyr Asp Ser Ile	
50	725 730 735 740	

	CAA ATC TAT GGT TAT GAG GGC CGG GGT TCC GTG GCT GGG TCC CTG AGC	2551	
	Gln Ile Tyr Gly Tyr Glu Gly Arg Gly Ser Val Ala Gly Ser Leu Ser		
	745	750	755
	TCC TTG GAG TCT GCC ACG ACA GAC TCA GAC CTG GAC TAC GAC TAT CTA	2599	
	Ser Leu Glu Ser Ala Thr Thr Asp Ser Asp Leu Asp Tyr Asp Tyr Leu		
	760	765	770
	CAG AAC TGG GGA CCT CGT TTT AAG AAA CTG GCA GAC TTG TAT GGC TCC	2647	
	Gln Asn Trp Gly Pro Arg Phe Lys Lys Leu Ala Asp Leu Tyr Gly Ser		
	775	780	785
	AAA GAC ACT TTT GAT GAT GAC TCT TAACAATAAT GTTACAAATT TGGCCTTAAG	2701	
	Lys Asp Thr Phe Asp Asp Asp Ser		
20	790	795	
	AACTGTGTCT GGCATTCTCA AGAACCTAGA AGATGTGTAACAGGTATTT TTTAAATCA	2761	
	AGGAAAGGCT CATTAAAAC AAGCAGAGTT TTACAGAGAG GAAACATTAA ATAACATGC	2821	
25	AAGGACATCA AAGTGGAAAA TACTGTGAAG TACCTTTCC CACTAAAAAA GCAAATATTG	2881	
	AAGTTGTTTA TCAACTTCAG TAGAAAAAAA AAAACCACTT GGCACACAAA ATATTTAAAT	2941	
	GAAGGAGAAG TCCACGGTGA ACTTACAATG AAGGGAAATC GTCTATGTGT TAAGAACATC	3001	
30	TAAGTCTCTC TTATTTTATT TTTAATTG TCAAAGAACCTTACCAACAAAA TTAGAAAGGA	3061	
	CAACAGTTCT GAGCTGAAAT TTCCACCTAA ACTATGGACA CTCTATCTGT AGTGCCTTT	3121	
	TAAACTTGA ATATATAATA TCCAGCCAGC TTAAACCCAT ACAATGTATG TACAATACAA	3181	
35	TGTACAATTAA TGTCTCTTGA GCATCAATCT TGTTACTGCT GATTCTTGTAAATCTTTTG	3241	
	CTTCTACTTT CATCCTAAAC TAATACGTGC CAGATATAAC TGTCTTGTCTT CAGTGAGGAG	3301	
	CACCCCTATTCTTCTGATT TTAAATGTAT CTATTTGTAC AATTTAAAG TTCTTATTT	3361	
40	AGTATACATA CAAATATCAG TATTCTGACA TGTACGAAAA TGTTACAGCA TCACACTTAT	3421	
	ATTTTATGAA CATTGTACTG TTGCTTTAAT ATGAGCTTCA ATATAAGAAG CAACTTTGA	3481	
	AATAAAAAAA AGATTCTTTT TTAAAAAAA AAAAAAAA AAAAAAAA AAAAAAAA	3541	
	AAAAAAA AAAAAAAA AAAAAAGCGG CGCGAATTG	3581	

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	CGA GAA GAG AGA GCC CAG TAC ACG TTG ATG GCT CAG GCG GTG GAC AGG	859		
	Arg Glu Glu Arg Ala Gln Tyr Thr Leu Met Ala Gln Ala Val Asp Arg			
5	120	125	130	
	GAC ACC AAT CGG CCA CTG GAG CCA CCG TCG GAA TTC ATT GTC AAG GTC	907		
	Asp Thr Asn Arg Pro Leu Glu Pro Pro Ser Glu Phe Ile Val Lys Val			
	135	140	145	
10	CAG GAC ATT AAT GAC AAC CCT CCG GAG TTC CTG CAC GAG ACC TAT CAT	955		
	Gln Asp Ile Asn Asp Asn Pro Pro Glu Phe Leu His Glu Thr Tyr His			
	150	155	160	165
	GCC AAC GTG CCT GAG AGG TCC AAT GTG GGA AGC TCA GTA ATC CAG GTG	1003		
	Ala Asn Val Pro Glu Arg Ser Asn Val Gly Thr Ser Val Ile Gln Val			
15	170	175	180	
	ACA GCT TCA GAT GCA GAT GAC CCC ACT TAT GGA AAT AGC GCC AAG TTA	1051		
	Thr Ala Ser Asp Ala Asp Asp Pro Thr Tyr Gly Asn Ser Ala Lys Leu			
	185	190	195	
20	GTG TAC AGT ATC CTC GAA GGA CAA CCC TAT TTT TCG GTG GAA GCA CAG	1099		
	Val Tyr Ser Ile Leu Glu Gly Gln Pro Tyr Phe Ser Val Glu Ala Gln			
	200	205	210	
25	ACA GGT ATC ATC AGA ACA GCC CTA CCC AAC ATG GAC AGG GAG GCC AAG	1147		
	Thr Gly Ile Ile Arg Thr Ala Leu Pro Asn Met Asp Arg Glu Ala Lys			
	215	220	225	
	GAG GAG TAC CAC GTG GTG ATC CAG GCC AAG GAC ATG GGT GGA CAT ATG	1195		
	Glu Glu Tyr His Val Val Ile Gln Ala Lys Asp Met Gly Gly His Met			
30	230	235	240	245
	GCC GGA CTC TCA GGG ACA ACC AAA GTG ACC ATC ACA CTG ACC GAT GTC	1243		
	Gly Gly Leu Ser Gly Thr Thr Lys Val Thr Ile Thr Leu Thr Asp Val			
	250	255	260	
35	AAT GAC AAC CCA CCA AAG TTT CCG CAG AGC GTA TAC CAG ATA TCT GTG	1291		
	Asn Asp Asn Pro Pro Lys Phe Pro Gln Ser Val Tyr Gln Ile Ser Val			
	265	270	275	
	TCA GAA GCA GCC GTC CCT GGG GAG GAA GTA GGA AGA GTG AAA GCT AAA	1339		
40	Ser Glu Ala Ala Val Pro Gly Glu Glu Val Gly Arg Val Lys Ala Lys			
	280	285	290	
	GAT CCA GAC ATT GGA GAA AAT GGC TTA GTC ACA TAC AAT ATT GTT GAT	1387		
	Asp Pro Asp Ile Gly Glu Asn Gly Leu Val Thr Tyr Asn Ile Val Asp			
45	295	300	305	
	GGA GAT GGT ATG GAA TCG TTT GAA ATC ACA ACC GAC TAT GAA ACA CAG	1435		
	Gly Asp Gly Met Glu Ser Phe Glu Ile Thr Thr Asp Tyr Glu Thr Gln			
	310	315	320	325
50				

	GAG GGG GTG ATA AAG CTG AAA AAG CCT GTA GAT TTT GAA ACC AAA AGA	1483
	Glu Gly Val Ile Lys Leu Lys Lys Pro Val Asp Phe Glu Thr Lys Arg	
5	330 335 340	
	GCC TAT AGC TTG AAG GTA GAG GCA AAC GTG CAC ATC GAC CCG AAG	1531
	Ala Tyr Ser Leu Lys Val Glu Ala Ala Asn Val His Ile Asp Pro Lys	
	345 350 355	
10	TTT ATC AGC AAT GGC CCT TTC AAG GAC ACT GTG ACC GTC AAG ATC GCA	1579
	Phe Ile Ser Asn Gly Pro Phe Lys Asp Thr Val Thr Val Lys Ile Ala	
	360 365 370	
	GTA GAA GAT GCT GAT GAG CCC CCT ATG TTC TTG GCC CCA AGT TAC ATC	1627
15	Val Glu Asp Ala Asp Glu Pro Pro Met Phe Leu Ala Pro Ser Tyr Ile	
	375 380 385	
	CAC GAA GTC CAA GAA AAT GCA GCT GCT GGC ACC GTG GTT GGG AGA GTG	1675
	His Glu Val Gln Glu Asn Ala Ala Ala Gly Thr Val Val Gly Arg Val	
20	390 395 400 405	
	CAT GCC AAA GAC CCT GAT GCT GCC AAC AGC CCG ATA AGG TAT TCC ATC	1723
	His Ala Lys Asp Pro Asp Ala Ala Asn Ser Pro Ile Arg Tyr Ser Ile	
	410 415 420	
25	GAT CGT CAC ACT GAC CTC GAC AGA TTT TTC ACT ATT AAT CCA GAG GAT	1771
	Asp Arg His Thr Asp Leu Asp Arg Phe Phe Thr Ile Asn Pro Glu Asp	
	425 430 435	
	GGT TTT ATT AAA ACT ACA AAA CCT CTG GAT AGA GAG GAA ACA GCC TGG	1819
30	Gly Phe Ile Lys Thr Thr Lys Pro Leu Asp Arg Glu Glu Thr Ala Trp	
	440 445 450	
	CTC AAC ATC ACT GTC TTT GCA GCA GAA ATC CAC AAT CGG CAT CAG GAA	1867
	Leu Asn Ile Thr Val Phe Ala Ala Glu Ile His Asn Arg His Gln Glu	
35	455 460 465	
	GCC AAA GTC CCA GTG GCC ATT AGG GTC CTT GAT GTC AAC GAT AAT GCT	1915
	Ala Lys Val Pro Val Ala Ile Arg Val Leu Asp Val Asn Asp Asn Ala	
	470 475 480 485	
40	CCC AAG TTT GCT GCC CCT TAT GAA GGT TTC ATC TGT GAG AGT GAT CAG	1963
	Pro Lys Phe Ala Ala Pro Tyr Glu Gly Phe Ile Cys Glu Ser Asp Gln	
	490 495 500	
	ACC AAG CCA CTT TCC AAC CAG CCA ATT GTT ACA ATT AGT GCA GAT GAC	2011
45	Thr Lys Pro Leu Ser Asn Gln Pro Ile Val Thr Ile Ser Ala Asp Asp	
	505 510 515	
	AAG GAT GAC ACG GCC AAT GGA CCA AGA TTT ATC TTC AGC CTA CCC CCT	2059
	Lys Asp Asp Thr Ala Asn Gly Pro Arg Phe Ile Phe Ser Leu Pro Pro	
50	520 525 530	

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	GAA ATC ATT CAC AAT CCA AAT TTC ACA GTC AGA GAC AAC CGA GAT AAC	2107
	Glut Ile Ile His Asn Pro Asn Phe Thr Val Arg Asp Asn Arg Asp Asn	
5	535 540 545	
	ACA GCA GGC GTG TAC GCC CGG CGT GGA GGG TTC AGT CGG CAG AAG CAG	2155
	Thr Ala Gly Val Tyr Ala Arg Arg Gly Gly Phe Ser Arg Gln Lys Gln	
	550 555 560 565	
10	GAC TTG TAC CTT CTG CCC ATA GTG ATC AGC GAT GGC GGC ATC CCG CCC	2203
	Asp Leu Tyr Leu Leu Pro Ile Val Ile Ser Asp Gly Gly Ile Pro Pro	
	570 575 580	
	ATG AGT AGC ACC AAC ACC CTC ACC ATC AAA GTC TGC GGG TGC GAC GTG	2251
	Met Ser Ser Thr Asn Thr Leu Thr Ile Lys Val Cys Gly Cys Asp Val	
15	585 590 595	
	AAC GGG GCA CTG CTC TCC AAC GCA GAG GGC TAC ATT CTG AAC GCC	2299
	Asn Gly Ala Leu Leu Ser Cys Asn Ala Glu Ala Tyr Ile Leu Asn Ala	
	600 605 610	
20	GGC CTG AGC ACA GGC GGC CTG ATC GGC ATC CTC GGC TGC ATC GTC ATT	2347
	Gly Leu Ser Thr Gly Ala Leu Ile Ala Ile Leu Ala Cys Ile Val Ile	
	615 620 625	
25	CTC CTG GTC ATT GTA GTA TTG TTT GTG ACC CTC AGA AGG CAA AAG AAA	2395
	Leu Leu Val Ile Val Val Leu Phe Val Thr Leu Arg Arg Gln Lys Lys	
	630 635 640 645	
	GAA CCA CTC ATT GTC TTT GAG GAA GAA GAT GTC CGT GAG AAC ATC ATT	2443
30	Glu Pro Leu Ile Val Phe Glu Glu Glu Asp Val Arg Glu Asn Ile Ile	
	650 655 660	
	ACT TAT GAT GAT GAA GGG GGT GGG GAA GAA GAC ACA GAA GCC TTT GAT	2491
	Thr Tyr Asp Asp Glu Gly Gly Glu Glu Asp Thr Glu Ala Phe Asp	
35	665 670 675	
	ATT GCC ACC CTC CAG AAT CCT GAT GGT ATC AAT GGA TTT ATC CCC CGC	2539
	Ile Ala Thr Leu Gln Asn Pro Asp Gly Ile Asn Gly Phe Ile Pro Arg	
	680 685 690	
40	AAA GAC ATC AAA CCT GAG TAT CAG TAC ATG CCT AGA CCT GGG CTC CGG	2587
	Lys Asp Ile Lys Pro Glu Tyr Gln Tyr Met Pro Arg Pro Gly Leu Arg	
	695 700 705	
	CCA GCG CCC AAC AGC GTG GAT GTC GAT GAC TTC ATC AAC ACG AGA ATA	2635
45	Pro Ala Pro Asn Ser Val Asp Val Asp Asp Phe Ile Asn Thr Arg Ile	
	710 715 720 725	
	CAG GAG GCA GAC AAT GAC CCC ACG GCT CCT CCT TAT GAC TCC ATT CAA	2683
	Gln Glu Ala Asp Asn Asp Pro Thr Ala Pro Pro Pro Tyr Asp Ser Ile Gln	
50	730 735 740	

	ATC TAC GGT TAT GAA GGC AGG GGC TCA GTG GCC GGG TCC CTG AGC TCC	2731	
	Ile Tyr Gly Tyr Glu Gly Arg Gly Ser Val Ala Gly Ser Leu Ser Ser		
5	745	750	755
	CTA GAG TCG GCC ACC ACA GAT TCA GAC TTG GAC TAT GAT TAT CTA CAG	2779	
	Leu Glu Ser Ala Thr Thr Asp Ser Asp Leu Asp Tyr Asp Tyr Leu Gln		
10	760	765	770
	AAC TGG GGA CCT CGT TTT AAG AAA CTA GCA GAT TTG TAT GGT TCC AAA	2827	
	Asn Trp Gly Pro Arg Phe Lys Lys Leu Ala Asp Leu Tyr Gly Ser Lys		
15	775	780	785
	GAC ACT TTT GAT GAC GAT TCT TAA CAATAACGAT ACAAAATTGG CCTTAAGAAC	2881	
	Asp Thr Phe Asp Asp Asp Ser		
20	790	795	
	TGTGTCTGGC GTTCTCAAGA ATCTAGAAGA TGTGAAACA GGTATTTTT TAAATCAAGG	2941	
	AAAGGCTCAT TTAAAAACAGG CAAAGTTTA CAGAGGAGT ACATTTAATA AACTGCGAG	3001	
	GACATCAAAG TGGTAAATAC TGTGAAATAC CTTTCTCAC AAAAAGGCAA ATATTGAAGT	3061	
25	TGTTTATCAA CTTCGCTAGA AAAAAAAAAC ACTTGGCATA CAAAATATT AAGTGAAGGA	3121	
	GAAGTCTAAC GCTGAACTGA CAATGAAGGG AAATTGTTA TGTGTTATGA ACATCCAAGT	3181	
	CTTTCTTCTT TTAAAGTTG TCAAAGAAC TTCCACAAAA TTAGAAAGGA CAACAGTTCT	3241	
	GAGCTGTAAT TTGCGCTTAA ACTCTGGACA CTCTATATGT AGTGCATTT TAAACTTGAA	3301	
30	ATATATAATA TTCAAGCCAGC TTAAACCCAT ACAATGTATG TACAATACAA TGTACAATTA	3361	
	TGTCTTTGA GCATCAATCT TGTTACTGCT GATTCTTGTA AATCTTTTG CTCTACTTT	3421	
	CATCTTAAAC TAATACGTGC CAGATATAAC TGTCTTGTGTT CAGTGAGAGA CGCCCTATTT	3481	
35	CTATGTCATT TTAAATGTAT CTATTTGTAC AATTTAAAG TTCTTATTT AGTATACATA	3541	
	TAAATATCAG TATTCTGACA TGTAAGAAAA TGTTACGGCA TCACACTTAT ATTTATGAA	3601	
	CATTGTACTG TTGCTTTAAT ATGAGCTTCA ATATAAGAAG CAATCTTGAA AATAAAAAAA	3661	
40	GATTTTTTT TAAAAAAAAG GAGATCTACA GGCGTGTAGA TCTCCGAATT C	3712	

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SEQ ID NO: 3
 SEQUENCE TYPE: nucleic acid
 SEQUENCE LENGTH: 3914 base pairs

STRANDEDNESS: double
 TOPOLOGY: linear
 MOLECULAR TYPE: cDNA to mRNA

ORIGINAL SOURCE
 ORGANISM: Homo sapiens
 TISSUE TYPE: osteosarcoma

FEATURE:
 SEQUENCE Description: SEQ ID NO: 3:

10	GAATTCGGAG ATCTACAGGC CCGCGACGCT CCCCTCAGCT GGCAGGGGCC GCGGAGAGAT	60
15	CCCGCGGGGG CGCGCTCGAG CGCGCGCTGA CTTGTGAATG GGACCGGGAC TGGGGCCGGG	120
20	ACTGACACCG CAGCGCTTGC CCTGCGCCAG GGACTGGCGG CTCGGAGGTT GCGTCCACCC	180
25	TCAAGGGCCC CAGAAATCAC TGTGTTTCA GCTCAGCGGC CCTGTGACAT TCCCTCGTGT	240
30	TGTCATTTGT TGAGTGACCA ATCAGATGGG TGGAGTGTGT TACAGAAATT GGCAGCAAGT	300
35	ATCCAATGGG TGAAGAAGAA GCTAACTGGG GACGTGGGCA GCGCTGACGT GATGAGCTCA	360
40	ACCACGAGAG ACATTCATC CCAAGAGAGG TCTGCGTGAC GCGTCCGGGA GCGCAACCTC	420
45	AGCAAGACCA CCGTACAGTT GGTGGAAAGGG GTGACAGCTG CATTCTCTG TGCCTACAC	480
50	GTAACCAAAA ATG AAG GAG AAC TAC TGT TTA CAA GCG GCC CTG GTG TGC	529
55	Met Lys Glu Asn Tyr Cys Leu Gln Ala Ala Leu Val Cys	
60	1 5 10	
65	CTG GGC ATG CTG TGC CAC AGC CAT GCC TTT GCG CCA GAG CCG CGG GGG	577
70	Leu Gly Met Leu Cys His Ser His Ala Phe Ala Pro Glu Arg Arg Gly	
75	15 20 25	
80	CAC CTG CGG CCC TCC TTC CAT GGG CAC CAT GAG AAG GGC AAG GAG GGG	625
85	His Leu Arg Pro Ser Phe His Gly His His Glu Lys Glu Lys Glu Gly	
90	30 35 40 45	
95	CAG GTG CTA CAG CGC TCC AAG CGT GGC TGG GTC TGG AAC CAG TTC TTC	673
100	Gln Val Leu Gln Arg Ser Lys Arg Gly Trp Val Trp Asn Gln Phe Phe	
105	50 55 60	
110	GTG ATA GAG GAG TAC ACC GGG CCT GAC CCC GTG CTT GTG GGC AGG CTT	721
115	Val Ile Glu Glu Tyr Thr Gly Pro Asp Pro Val Leu Val Gly Arg Leu	
120	65 70 75	
125	CAT TCA GAT ATT GAC TCT GGT GAT GGG AAC ATT AAA TAC ATT CTC TCA	769
130	His Ser Asp Ile Asp Ser Gly Asp Gly Asn Ile Lys Tyr Ile Leu Ser	
135	80 85 90	
140	GGG GAA GGA GCT GGA ACC ATT TTT GTG ATT GAT GAC AAA TCA GGG AAC	817
145	Gly Glu Gly Ala Gly Thr Ile Phe Val Ile Asp Asp Lys Ser Gly Asn	

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	ATT CAT GCC ACC AAG ACG TTG GAT CGA GAA GAG AGA GCC CAG TAC ACG	865
	Ile His Ala Thr Lys Thr Leu Asp Arg Glu Glu Arg Ala Gln Tyr Thr	
5	110 115 120 125	
	TTG ATG GCT CAG GCG GTG GAC AGG GAC ACC AAT CGG CCA CTG GAG CCA	913
	Leu Met Ala Gln Ala Val Asp Arg Asp Thr Asn Arg Pro Leu Glu Pro	
	130 135 140	
10	CCG TCG GAA TTC ATT GTC AAG GTC CAG GAC ATT AAT GAC AAC CCT CCG	961
	Pro Ser Glu Phe Ile Val Lys Val Gln Asp Ile Asn Asp Asn Pro Pro	
	145 150 155	
	GAG TTC CTG CAC GAG ACC TAT CAT GCC AAC GTG CCT GAG AGG TCC AAT	1009
	Glu Phe Leu His Glu Thr Tyr His Ala Asn Val Pro Glu Arg Ser Asn	
15	160 165 170	
	GTG GGA ACG TCA GTA ATC CAG GTG ACA GCT TCA GAT GCA GAT GAC CCC	1057
	Val Gly Thr Ser Val Ile Gln Val Thr Ala Ser Asp Ala Asp Asp Pro	
	175 180 185	
20	ACT TAT GGA AAT AGC AAC TTA GTG TAC AGT ATC CTC GAA GGA CAA	1105
	Thr Tyr Gly Asn Ser Ala Lys Leu Val Tyr Ser Ile Leu Glu Gly Gln	
	190 195 200 205	
25	CCC TAT TTT TCG GTG GAA GCA CAG ACA GGT ATC ATC AGA ACA GCA CTA	1153
	Pro Tyr Phe Ser Val Glu Ala Gln Thr Gly Ile Ile Arg Thr Ala Leu	
	210 215 220	
	CCC AAC ATG GAC AGG GAG GCC AAG GAG GAG TAC CAC GTG GTG ATC CAG	1201
30	Pro Asn Met Asp Arg Glu Ala Lys Glu Glu Tyr His Val Val Ile Gln	
	225 230 235	
	GCC AAG GAC ATG GGT GGA CAT ATG GCC GGA CTC TCA GGG ACA ACC AAA	1249
	Ala Lys Asp Met Gly Gly His Met Gly Gly Leu Ser Gly Thr Thr Lys	
35	240 245 250	
	GTG ACG ATC ACA CTG ACC GAT GTC AAT GAC AAC CCA CCA AAG TTT CCG	1297
	Val Thr Ile Thr Leu Thr Asp Val Asn Asp Asn Pro Pro Lys Phe Pro	
	255 260 265	
40	CAG AGC GTA TAC CAG ATA TCT GTG TCA GAA GCA GCC GTC CCT GGG GAG	1345
	Gln Ser Val Tyr Gln Ile Ser Val Ser Glu Ala Ala Val Pro Gly Glu	
	270 275 280 285	
	GAA GTA GGA AGA GTG AAA GCT AAA GAT CCA GAC ATT GGA GAA AAT GGC	1393
45	Glu Val Gly Arg Val Lys Ala Lys Asp Pro Asp Ile Gly Glu Asn Gly	
	290 295 300	
	TTA GTC ACA TAC AAT ATT GTT GAT GGA GAT GGT ATG GAA TCG TTT GAA	1441
	Leu Val Thr Tyr Asn Ile Val Asp Gly Asp Gly Met Glu Ser Phe Glu	
50	305 310 315	

	ATC ACA ACG GAC TAT GAA ACA CAG GAG GGG GTG ATA AAG CTG AAA AAG	1489
	Ile Thr Thr Asp Tyr Glu Thr Gln Glu Gly Val Ile Lys Leu Lys Lys	
5	320 325 330	
	CCT GTA GAT TTT GAA ACC AAA AGA GCC TAT AGC TTG AAG GTA CAG GCA	1537
	Pro Val Asp Phe Glu Thr Lys Arg Ala Tyr Ser Leu Lys Val Glu Ala	
	335 340 345	
10	GCC AAC GTG CAC ATC GAC CCG AAG TTT ATC AGC AAT GGC CCT TTC AAG	1585
	Ala Asn Val His Ile Asp Pro Lys Phe Ile Ser Asn Gly Pro Phe Lys	
	350 355 360 365	
	GAC ACT GTG ACC GTC AAG ATC GCA GTA GAA GAT GCT GAT GAG CCC CCT	1633
	Asp Thr Val Thr Val Lys Ile Ala Val Glu Asp Ala Asp Glu Pro Pro	
15	370 375 380	
	ATG TTC TTG GCC CCA AGT TAC ATC CAC GAA GTC CAA GAA AAT GCA GCT	1681
	Met Phe Leu Ala Pro Ser Tyr Ile His Glu Val Gln Glu Asn Ala Ala	
	385 390 395	
20	GCT GGC ACC GTG GTT GGG AGA GTG CAT GCC AAA GAC CCT GAT GCT GCC	1729
	Ala Gly Thr Val Val Gly Arg Val His Ala Lys Asp Pro Asp Ala Ala	
	400 405 410	
25	AAC AGC CCG ATA AGG TAT TCC ATC GAT CGT CAC ACT GAC CTC GAC AGA	1777
	Asn Ser Pro Ile Arg Tyr Ser Ile Asp Arg His Thr Asp Leu Asp Arg	
	415 420 425	
	TTT TTC ACT ATT AAT CCA GAG GAT GGT TTT ATT AAA ACT ACA AAA CCT	1825
	Phe Phe Thr Ile Asn Pro Glu Asp Gly Phe Ile Lys Thr Thr Lys Pro	
30	430 435 440 445	
	CTG GAT AGA GAG GAA ACA GCC TGG CTC AAC ATC ACT GTC TTT GCA GCA	1873
	Leu Asp Arg Glu Glu Thr Ala Trp Leu Asn Ile Thr Val Phe Ala Ala	
	450 455 460	
35	GAA ATC CAC AAT CGG CAT CAG GAA GCC AAA GTC CCA GTG GCC ATT AGG	1921
	Glu Ile His Asn Arg His Gln Glu Ala Lys Val Pro Val Ala Ile Arg	
	465 470 475	
40	GTC CTT GAT GTC AAC GAT AAT GCT CCC AAG TTT GCT GCC CCT TAT GAA	1969
	Val Leu Asp Val Asn Asp Ala Pro Lys Phe Ala Ala Pro Tyr Glu	
	480 485 490	
45	GGT TTC ATC TGT GAG AGT GAT CAG ACC AAG CCA CTT TCC AAC CAG CCA	2017
	Gly Phe Ile Cys Glu Ser Asp Gln Thr Lys Pro Leu Ser Asn Gln Pro	
	495 500 505	
	ATT GTT ACA ATT AGT GCA GAT GAC AAG GAT GAC AGC GGC AAT GGA CCA	2065
	Ile Val Thr Ile Ser Ala Asp Asp Lys Asp Asp Thr Ala Asn Gly Pro	
50	510 515 520 525	

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	AGA TTT ATC TTC AGC CTA CCC CCT GAA ATC ATT CAC AAT CCA AAT TTC	2113
	Arg Phe Ile Phe Ser Leu Pro Pro Glu Ile Ile His Asn Pro Asn Phe	
	530 535 540	
5	ACA GTC AGA GAC AAC CGA GAT AAC ACA GCA GGC GTG TAC GCC CGG CGT	2161
	Thr Val Arg Asp Asn Arg Asp Asn Thr Ala Gly Val Tyr Ala Arg Arg	
	545 550 555	
10	GGA GGG TTC AGT CGG CAG AAG CAG GAC TTG TAC CTT CTG CCC ATA GTG	2209
	Gly Gly Phe Ser Arg Gln Lys Asp Leu Tyr Leu Leu Pro Ile Val	
	560 565 570	
	ATC AGC GAT GGC GGC ATC CCG CCC ATG AGT AGC ACC AAC ACC CTC ACC	2257
	Ile Ser Asp Gly Gly Ile Pro Pro Met Ser Ser Thr Asn Thr Leu Thr	
15	575 580 585	
	ATC AAA GTC TGC GGG TGC GAC GTG AAC GGG GCA CTG CTC TCC TGC AAC	2305
	Ile Lys Val Cys Gly Cys Asp Val Asn Gly Ala Leu Leu Ser Cys Asn	
	590 595 600 605	
20	GCA GAG GCC TAC ATT CTG AAC GGC CTG AGC ACA GGC GCC CTG ATC	2353
	Ala Glu Ala Tyr Ile Leu Asn Ala Gly Leu Ser Thr Gly Ala Leu Ile	
	610 615 620	
	GCC ATC CTC GCC TGC ATC GTC ATT CTC CTG GGT TGC CCA AGC TTA ATG	2401
25	Ala Ile Leu Ala Cys Ile Val Ile Leu Leu Gly Cys Pro Ser Leu Met	
	625 630 635	
	GAA CCC CCC TCT CCC AGG GAA GAC ATG AGA TTG CTT TAT CTG GGC TTC	2449
	Glu Pro Pro Ser Pro Arg Glu Asp Met Arg Leu Leu Tyr Leu Gly Phe	
30	640 645 650	
	CAG CTG ATG CTA TTT TCC TAT GTT AAA GAA AAC AGA AGA TTT TGT CTT	2497
	Gln Leu Met Leu Phe Ser Tyr Val Lys Val Asn Arg Arg Phe Cys Leu	
	655 660 665	
35	CTG GGG GTC TTT ATA AAA CCT CCT TTC CTC TAT GTG GTG GCT ACA GAG	2545
	Leu Gly Val Phe Ile Lys Leu Pro Phe Leu Tyr Val Val Ala Thr Glu	
	670 675 680 685	
40	AGT CCA ACC ACA CTT ACG TCA TTG TAGTATTGTT TGTGACCCCTG AGAAGGCAAA	2599
	Ser Pro Thr Thr Leu Thr Ser Leu	
	690	
	AGAAAAGAACC ACTCATGTC TTTGAGGAAG AAGATGTCCG TGAGAACATC ATTACTTATG	2659
45	ATGATGAAGG GGGTGGGGAA GAAGACACAG AAGCCTTGTG TATTGCCACC CTCCAGAAC	2719
	CTGATGGTAT CAATGGATT ATCCCCCGCA AAGACATCAA ACCTGAGTAT CAGTACATGC	2779
	CTAGACCTGG GCTCCGGCCA GCGCCCAACA GCGTGGATGT CGATGACTTC ATCAACACGA	2839
	GAATACAGGA GGCAGACAAAT GACCCCACGG CTCTCCCTTA TGACTCCATT CAAATCTACG	2899
50	GTATGAAGG CAGGGGCTCA GTGGCCGGGT CCTTGAGCTC CCTAGAGTCG GCCACCAAG	2959

5	ATTCAGACTT GGACTATGAT TATCTACAGA ACTGGGGACC TCGTTTTAAG AAACTAGCAG	3019
	ATTTGTATGG TTCCAAAGAC ACTTTGATG ACGATTCTTA ACAATAACGA TACAAATTG	3079
	GCCTTAAGAA CTGTGTCTGG CGTTCTCAAG AATCTAGAAG ATGTGTAAAC AGGTATTTT	3139
10	TTAAATCAAG GAAAGGCTCA TTTAAAACAG GCAAAGTTT ACAGAGAGGA TACATTTAAT	3199
	AAAACCTGCGA GGACATCAA GTGGTAAATA CTGTGAAATA CCTTTCTCA CAAAAGGCA	3259
	AATATTGAAG TTGTTTATCA ACTTCGCTAG AAAAAAAA CACTTGGCAT AAAAAATATT	3319
	TAAGTGAAGG AGAAGTCTAA CGCTGAAGT ACAATGAAGG GAAATTGTT ATGTGTTATG	3379
15	AAACATCCAAG TCTTTCTCT TTTTAAGTT GTCAAAGAAG CTTCCACAAA ATTAGAAAGG	3439
	ACAACAGTTC TGAGCTGTAA TTTGCCCTTA AACTCTGGAC ACTCTATATG TAGTGCATT	3499
	TTAAACTTGA AATATATAAT ATTCAGCCAG CTTAAACCCA TACAATGTAT GTACAATACA	3559
	ATGTACAATT ATGTCTCTTGT AGCATCAATC TTGTTACTGC TGATTCTTGT AAATCTTTT	3619
20	GCTTCTACTT TCATCTTAAA CTAATACGTG CCAGATATAA CTGTCTGTT TCAGTGAGAG	3679
	ACGCCCTATT TCTATGTCA TTTTAATGTA TCTATTTGTA CAATTTAAA GTTCTTATT	3739
	TAGTATAACAT ATAAATATCA GTATTCTGAC ATGTAAGAAA ATGTTACGGC ATCACACTTA	3799
	TATTTTATGA ACATTGTACT GTTGCTTTAA TATGAGCTTC AATATAAGAA GCAATCTTG	3859
25	AAATAAAAAAA AGATTTTTT TTCCGGAGATC TACAGGCCGT TAGATCTCCG AATTC	3914

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SEQ ID NO: 4
SEQUENCE TYPE: nucleic acid
SEQUENCE LENGTH: 21 base pairs

5 STRANDEDNESS: single
TOPOLOGY: linear
MOLECULAR TYPE: other nucleic acid

ORIGINAL SOURCE
ORGANISM: none
STRAIN: none

10 FEATURE: linker DNA with sequence complementary to SEQ ID NO: 5, termed "ATOS-1"
SEQUENCE Description: SEQ ID NO: 4:

CTCTTGCTTG AATTCGGACT A 21

15 SEQ ID NO: 5
SEQUENCE TYPE: nucleic acid
SEQUENCE LENGTH: 25 base pairs

STRANDEDNESS: single
TOPOLOGY: linear
MOLECULAR TYPE: other nucleic acid

20 ORIGINAL SOURCE
ORGANISM: none
STRAIN: none
FEATURE: linker DNA with sequence complementary to SEQ ID NO: 4, termed "ATOS-2"
SEQUENCE Description: SEQ ID NO: 5:

25 TAGTCCGAAT TCAAGCAAGA GCACA 25

30 SEQ ID NO: 6
SEQUENCE TYPE: nucleic acid
SEQUENCE LENGTH: 21 base pairs

STRANDEDNESS: single
TOPOLOGY: linear
MOLECULAR TYPE: other nucleic acid

35 ORIGINAL SOURCE
ORGANISM: none
STRAIN: none
FEATURE: linker DNA with sequence complementary to SEQ ID NO: 7, termed "ATOS-4"
SEQUENCE Description: SEQ ID NO: 6:

CTCTTGCTTA AGCTTGGACT A 21

40 SEQ ID NO: 7
SEQUENCE TYPE: nucleic acid
SEQUENCE LENGTH: 25 base pairs

STRANDEDNESS: single
TOPOLOGY: linear
MOLECULAR TYPE: other nucleic acid

45 ORIGINAL SOURCE
ORGANISM: none
STRAIN: none
FEATURE: linker DNA with sequence complementary to SEQ ID NO: 6, termed "ATOS-5"
SEQUENCE Description: SEQ ID NO: 7:

50 TAGTCCAAGC TTAAGCAAGA GCACA 25

SEQ ID NO: 8
 SEQUENCE TYPE: amino acid
 SEQUENCE LENGTH: 15 amino acids

TOPOLOGY: linear
 MOLECULAR TYPE: peptide

ORIGINAL SOURCE
 ORGANISM: *Mus musculus*

FEATURE: OSF-4.1 (antigen peptide)

LOCATION:

segment of mouse OSF-4 from the 101st to the 115th amino acid residue
 SEQUENCE Description: SEQ ID NO: 8:

Phe Val Ile Asp Asp Lys Ser Gly Asn Ile His Ala Thr Lys Thr
 5 10 15

Claims

20. 1. A protein comprising mOSF-4 having an amino acid sequence at the 25th to 796th positions in Sequence ID No. 1 of the Sequence Listing, hOSF-4-1 having an amino acid sequence at the 25th to 796th positions in Sequence ID No. 2 of the Sequence Table, or hOSF-4-2 having an amino acid sequence at the 25th to 693rd positions in Sequence ID No. 3 of the Sequence Listing; or an analogue of the mOSF-4, hOSF-4-1 or hOSF-4-2; or a fragment of the mOSF-4, hOSF-4-1 or hOSF-4-2.
25. 2. A protein comprising mOSF-4 precursor protein having an amino acid sequence at the 1st to 796th positions, including a signal peptide at the 1st to 24th positions, in Sequence ID No. 1 of the Sequence Listing, hOSF-4-1 precursor protein having an amino acid sequence at the 1st to 796th positions, including a signal peptide at the 1st to 24th positions, in Sequence ID No. 2 of the Sequence Listing, or hOSF-4-2 precursor protein having an amino acid sequence at the 1st to 693rd positions, including a signal peptide at the 1st to 24th positions, in Sequence ID No. 3 of the Sequence Listing; or an analogue of each precursor protein; or a fragment of each precursor protein.
30. 3. DNA or RNA coding for the protein of Claim 1 or 2.
35. 4. DNA or RNA hybridizing under stringent conditions with DNA or RNA according to claim 3.
5. 5. A process for the production of a recombinant mammalian OSF-4 protein according to claim 1, or an analogue thereof, or a fragment thereof, comprising the steps of:
 40. (a) obtaining a population of cells containing a heterogeneous DNA composed of the following DNA sequences:
 - (i) a sequence which can function in the cells to control transcription and translation, and
 - (ii) a DNA sequence joined downstream of said controlling sequence to code for said recombinant protein, and
 45. (b) culturing said population of cells under conditions which permit the production of said recombinant protein.
6. The process of Claim 5, wherein the controlling sequence further contains a DNA coding for a signal peptide for secreting said recombinant protein extracellularly such that said DNA is positioned immediately upstream of said DNA sequence coding for said recombinant protein.
50. 7. The process of Claim 5 or 6, wherein the population of cells is *Escherichia coli*, or yeast, or mammalian cells.
55. 8. A diagnostic reagent for bone metabolic diseases, containing the whole or a fragment of the DNA or RNA of Claim 3 or 4.
9. A diagnostic reagent for bone metabolic diseases, containing the protein of Claim 1.

10. A polyclonal or monoclonal antibody against the protein of Claim 1.
11. A diagnostic reagent for bone metabolic diseases, containing the antibody of Claim 10.
- 5 12. A therapeutic agent for bone metabolic diseases, containing the protein of Claim 1.

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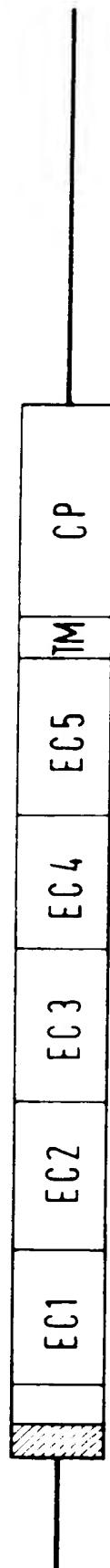


Fig. 1

Fig. 2

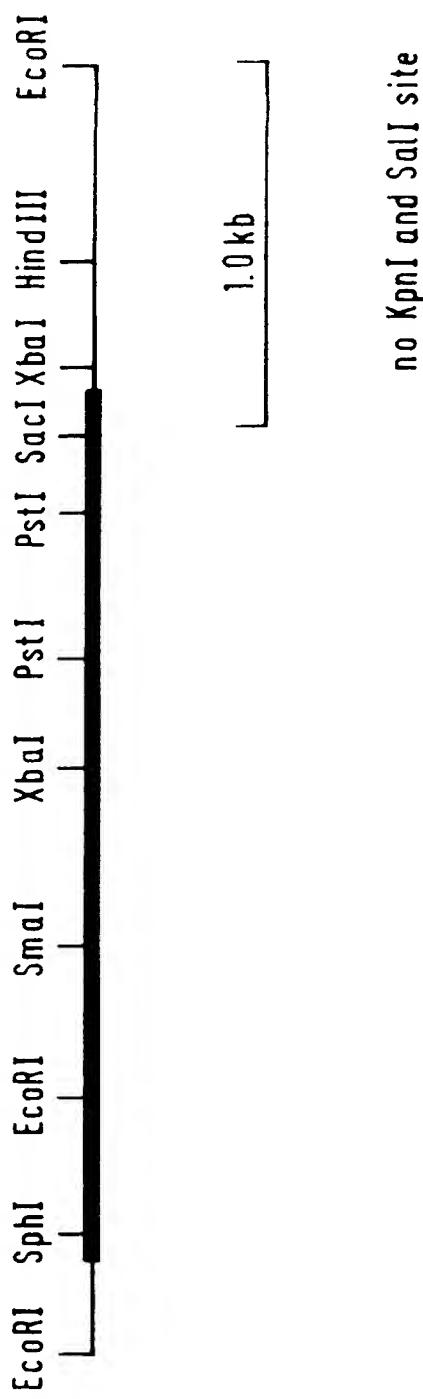
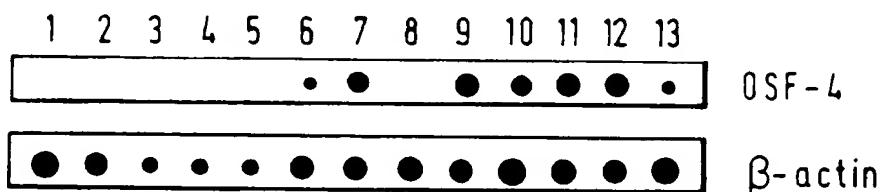


Fig. 3

- 1 THYMUS
- 2 SPLEEN
- 3 BRAIN
- 4 KIDNEY
- 5 LIVER
- 6 LUNG
- 7 TESTIS
- 8 HEART
- 9 OSTEOBLAST-ENRICHED CELL FROM MOUSE CALVARIA
- 10 MC3T3-E1 CELLS FROM 3 DAYS CULTURE
- 11 MC3T3-E1 CELLS FROM 12 DAYS CULTURE
- 12 MC3T3-E1 CELLS FROM 60 DAYS CULTURE
- 13 NIH3T3 CELLS

Fig. 4

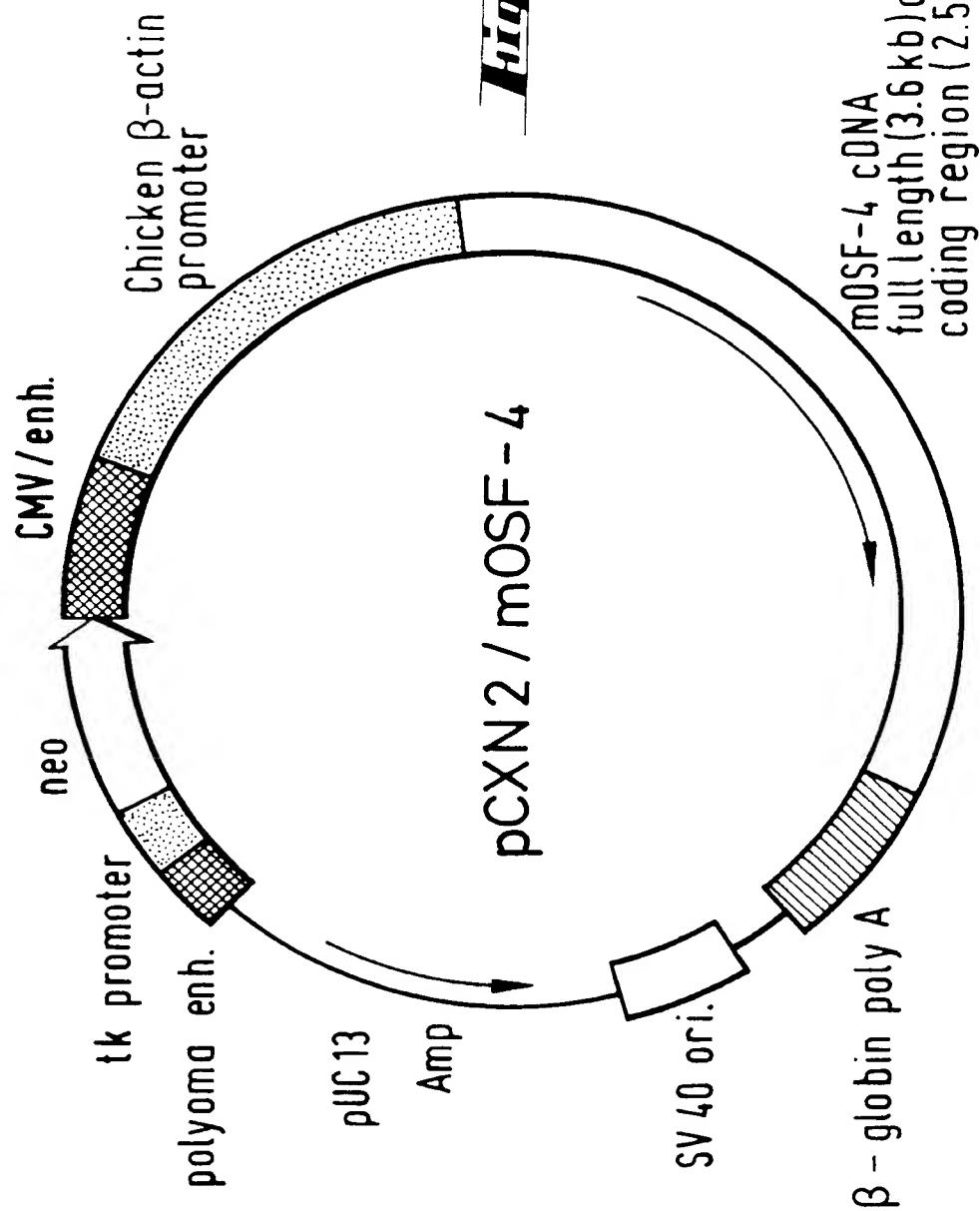


Table 5: Aggregation Assay of the cloned cell lines expressing mouse OSF-4

Cell lines	No. of cell clots after 1 hr (N1)/ No. of cells pre-stirring (N0)	
	with 1 mM Ca ²⁺	without 1 mM Ca ²⁺
TC-treated		
L-cell (Control)	1.00(*1)	1.00
C1	0.65	1.00
C7	0.29	1.00
C11	0.51	1.00
mock(*2)	1.00	1.00
TE-treated		
C7	1.00	1.00

(*1) The smaller the number of cell clots after one-hour stirring, the stronger cell aggregation occurred. The number 1.00 means no aggregation.

(*2) "mock" refers to the control cell into which the vector alone was introduced.

OSF-4 provided by the present invention can be used as an agent for treating bone metabolic diseases, and its high organ specificity for bones enables its use as a diagnostic reagent for bone metabolic diseases.

[Table 1]

		5	10	15	20	25	30	35	40	45	50
1	MKENYCLQAA	LYCLSMYHS	QAFALERSH	LPSFGHHE	KGKEGAVLQR	SKRGWVANQF	FVIEEYTPD	PVLVGRHLSD	DSGGMNHY	ILSGEGAGT	100
hosF-4	MKENYCLQAA	LVLGMLCHS	HAFAPERCH	LPSFGHHE	KGKEGAVLQR	SKRGWVANQF	FVIEEYTPD	PVLVGRHLSD	DSGGMNHY	ILSGEGAGT	
hosF-4-1	MKENYCLQAA	LVLGMLCHS	HAFAPERCH	LPSFGHHE	KGKEGAVLQR	SKRGWVANQF	FVIEEYTPD	PVLVGRHLSD	DSGGMNHY	ILSGEGAGT	
hosF-4-2	MKENYCLQAA	LVLGMLCHS	HAFAPERCH	LPSFGHHE	KGKEGAVLQR	SKRGWVANQF	FVIEEYTPD	PVLVGRHLSD	DSGGMNHY	ILSGEGAGT	
Consensus	MKENYCLQAA	LYCL-ML-HS	-AF-ERR-H	L-PSFGHHE	KGKEGAVLQR	SKRGWVANQF	FVIEEYTPD	PVLVGRHLSD	DSGGMNHY	ILSGEGAGT	
101	FVDDDKSGM1	HATKLDRE	RAQYLMQA	VDRTMRPL	PPSEFIVKQ	DINDPPEFL	HEIYHANPE	RSNVGTSVQ	VTASADDPT	YGSAKLVS	200
hosF-4	FVDDDKSGM1	HATKLDRE	RAQYLMQA	VDRTMRPL	PPSEFIVKQ	DINDPPEFL	HEIYHANPE	RSNVGTSVQ	VTASADDPT	YGSAKLVS	
hosF-4-1	FVDDDKSGM1	HATKLDRE	RAQYLMQA	VDRTMRPL	PPSEFIVKQ	DINDPPEFL	HEIYHANPE	RSNVGTSVQ	VTASADDPT	YGSAKLVS	
hosF-4-2	FVDDDKSGM1	HATKLDRE	RAQYLMQA	VDRTMRPL	PPSEFIVKQ	DINDPPEFL	HEIYHANPE	RSNVGTSVQ	VTASADDPT	YGSAKLVS	
Consensus	FVDDDKSGM1	HATKLDRE	RAQYLMQA	VDRTMRPL	PPSEFIVKQ	DINDPPEFL	HEIYHANPE	RSNVGTSVQ	VTASADDPT	YGSAKLVS	
201	ILEGQPFYV	EAQTGILRITA	LPMOREAKE	ETHVVIQAKD	MGGHMGGLSG	TTKVITLTD	VNDNPKFPQ	SYQHSSYSEA	AVPGEEVGRV	KADPOIGEN	300
hosF-4	ILEGQPFYV	EAQTGILRITA	LPMOREAKE	ETHVVIQAKD	MGGHMGGLSG	TTKVITLTD	VNDNPKFPQ	SYQHSSYSEA	AVPGEEVGRV	KADPOIGEN	
hosF-4-1	ILEGQPFYV	EAQTGILRITA	LPMOREAKE	ETHVVIQAKD	MGGHMGGLSG	TTKVITLTD	VNDNPKFPQ	SYQHSSYSEA	AVPGEEVGRV	KADPOIGEN	
hosF-4-2	ILEGQPFYV	EAQTGILRITA	LPMOREAKE	ETHVVIQAKD	MGGHMGGLSG	TTKVITLTD	VNDNPKFPQ	SYQHSSYSEA	AVPGEEVGRV	KADPOIGEN	
Consensus	ILEGQPFYV	EAQTGILRITA	LPMOREAKE	ETHVVIQAKD	MGGHMGGLSG	TTKVITLTD	VNDNPKFPQ	SYQHSSYSEA	AVPGEEVGRV	KADPOIGEN	
301	GLVTTWIVDG	DGIELEFITT	DYETQDGWK	LKRPVDFETK	RAYSLKIEA	NWHDPKFIS	MGPFDOTVY	KISVEDADEF	PMFLAPSYTH	EVQENAAAGT	400
hosF-4	GLVTTWIVDG	DGMESEITI	DYETQDGWK	LKRPVDFETK	RAYSLKIEA	NWHDPKFIS	MGPFDOTVY	KIAVEDADEF	PMFLAPSYTH	EVQENAAAGT	
hosF-4-1	GLVTTWIVDG	DGMESEITI	DYETQDGWK	LKRPVDFETK	RAYSLKIEA	NWHDPKFIS	MGPFDOTVY	KIAVEDADEF	PMFLAPSYTH	EVQENAAAGT	
hosF-4-2	GLVTTWIVDG	DGMESEITI	DYETQDGWK	LKRPVDFETK	RAYSLKIEA	NWHDPKFIS	MGPFDOTVY	KI-VEDADEF	PMFLAPSYTH	EVQENAAAGT	
Consensus	GLVTTWIVDG	DG-E-FEITT	DYETQ-DV-K	LKRPVDFETK	RAYSLK-EA	NWHDPKFIS	MGPFDOTVY	KI-VEDADEF	PMFLAPSYTH	EVQENAAAGT	
401	YVGRVHAKD	DAANSPIRYS	IDRNTLDRF	FTINPEDGEI	KITKPLDRE	TAMLNISFA	AETIHRHQET	KVPAIRYLD	VNDNPKFIA	PYEGFICED	500
hosF-4	YVGRVHAKD	DAANSPIRYS	IDRNTLDRF	FTINPEDGEI	KITKPLDRE	TAMLNITFA	AETIHRHQEA	KVPAIRYLD	VNDNPKFIA	PYEGFICED	
hosF-4-1	YVGRVHAKD	DAANSPIRYS	IDRNTLDRF	FTINPEDGEI	KITKPLDRE	TAMLNITFA	AETIHRHQEA	KVPAIRYLD	VNDNPKFIA	PYEGFICED	
hosF-4-2	YVGRVHAKD	DAANSPIRYS	IDRNTLDRF	FTINPEDGEI	KITKPLDRE	TAMLNITFA	AETIHRHQEA	KVPAIRYLD	VNDNPKFIA	PYEGFICED	
Consensus	YVGRVHAKD	DAANSPIRYS	IDRNTLDRF	FTINPEDGEI	KITKPLDRE	TAMLNITFA	AETIHRHQEA	KVPAIRYLD	VNDNPKFIA	PYEGFICED	
501	HPKALSNOP1	VTYSADDODD	TANGPRFIF	LPPE1HMPN	FTYRDNRNT	AGYARGGF	SRQK0DYL	PIVISDGIP	PMSSNTLTI	KVCGCOWNGA	600
hosF-4	HPKALSNOP1	VTYSADDODD	TANGPRFIF	LPPE1HMPN	FTYRDNRNT	AGYARGGF	SRQK0DYL	PIVISDGIP	PMSSNTLTI	KVCGCOWNGA	
hosF-4-1	HPKALSNOP1	VTYSADDODD	TANGPRFIF	LPPE1HMPN	FTYRDNRNT	AGYARGGF	SRQK0DYL	PIVISDGIP	PMSSNTLTI	KVCGCOWNGA	
hosF-4-2	HPKALSNOP1	VTYSADDODD	TANGPRFIF	LPPE1HMPN	FTYRDNRNT	AGYARGGF	SRQK0DYL	PIVISDGIP	PMSSNTLTI	KVCGCOWNGA	
Consensus	-K-LSNOP1	VT-SADD-D0	TANGPRFIF	LPPE1-HMPN	FTYRDNRNT	AGYARGGF	SRQK0DYL	PIVISDGIP	PMSSNTLTI	KVCGCOWNGA	

[Table 2]

		5	10	15	20	25	30	35	40	45	50
1	50	SKRGWVANQF	FVIEEYTPD	PVLVGRHLSD	DSGGMNHY	ILSGEGAGT					
hosF-4	50	SKRGWVANQF	FVIEEYTPD	PVLVGRHLSD	DSGGMNHY	ILSGEGAGT					
hosF-4-1	50	SKRGWVANQF	FVIEEYTPD	PVLVGRHLSD	DSGGMNHY	ILSGEGAGT					
hosF-4-2	50	SKRGWVANQF	FVIEEYTPD	PVLVGRHLSD	DSGGMNHY	ILSGEGAGT					
Consensus	50	SKRGWVANQF	FVIEEYTPD	PVLVGRHLSD	DSGGMNHY	ILSGEGAGT					
101	150	PPSEFIVKQ	DINDPPEFL	HEIYHANPE	RSNVGTSVQ	VTASADDPT	YGSAKLVS				
hosF-4	150	PPSEFIVKQ	DINDPPEFL	HEIYHANPE	RSNVGTSVQ	VTASADDPT	YGSAKLVS				
hosF-4-1	150	PPSEFIVKQ	DINDPPEFL	HEIYHANPE	RSNVGTSVQ	VTASADDPT	YGSAKLVS				
hosF-4-2	150	PPSEFIVKQ	DINDPPEFL	HEIYHANPE	RSNVGTSVQ	VTASADDPT	YGSAKLVS				
Consensus	150	PPSEFIVKQ	DINDPPEFL	HEIYHANPE	RSNVGTSVQ	VTASADDPT	YGSAKLVS				
201	250	250	250	250	250	250	250	250	250	250	250
hosF-4	250	250	250	250	250	250	250	250	250	250	250
hosF-4-1	250	250	250	250	250	250	250	250	250	250	250
hosF-4-2	250	250	250	250	250	250	250	250	250	250	250
Consensus	250	250	250	250	250	250	250	250	250	250	250
301	350	350	350	350	350	350	350	350	350	350	350
hosF-4	350	350	350	350	350	350	350	350	350	350	350
hosF-4-1	350	350	350	350	350	350	350	350	350	350	350
hosF-4-2	350	350	350	350	350	350	350	350	350	350	350
Consensus	350	350	350	350	350	350	350	350	350	350	350
401	450	450	450	450	450	450	450	450	450	450	450
hosF-4	450	450	450	450	450	450	450	450	450	450	450
hosF-4-1	450	450	450	450	450	450	450	450	450	450	450
hosF-4-2	450	450	450	450	450	450	450	450	450	450	450
Consensus	450	450	450	450	450	450	450	450	450	450	450
501	550	550	550	550	550	550	550	550	550	550	550
hosF-4	550	550	550	550	550	550	550	550	550	550	550
hosF-4-1	550	550	550	550	550	550	550	550	550	550	550
hosF-4-2	550	550	550	550	550	550	550	550	550	550	550
Consensus	550	550	550	550	550	550	550	550	550	550	550



EUROPEAN PATENT APPLICATION

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29.06.94 Bulletin 94/26(8) Applicant: HOECHST JAPAN LIMITED
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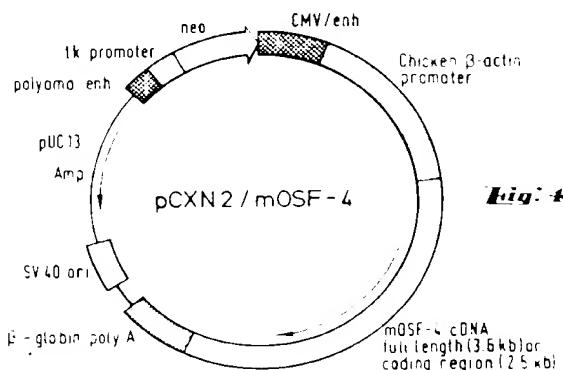
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(11) Bone-related cadherin-like protein and process for its production.

(12) A bone-related protein named OSF-4 which is obtained from bone tissue of a mammal including mouse or human, and a process for its production. This protein is a novel naturally occurring mammal protein of the cadherin family.

OSF-4 acts as an adhesion molecule or a growth factor which takes part in the process of osteogenesis at the site of bone induction. OSF-4 can be used as an agent for treating bone metabolic diseases, and its high organ specificity for bones enables its use as a diagnostic reagent for bone metabolic diseases.





European Patent
Office

EUROPEAN SEARCH REPORT

Application Number
EP 93 11 3602

DOCUMENTS CONSIDERED TO BE RELEVANT					
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.CLS)		
A	BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS. vol. 185, no. 1 , 29 May 1992 , DULUTH, MINNESOTA US pages 224 - 230 'Takamatsu H; Itoh M; Kimura M; Gospodarowicz D; Amann E; "Expression and purification of biologically active human OSF-1 in Escherichia coli"' * the whole document * ---	1-12	C12N15/12 C07K13/00 G01N33/68 C07K15/28 C12P21/08 A61K39/395 G01N33/577 A61K37/02		
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X	GENOMICS vol. 12 , 1992 pages 517 - 525 BOYLE, A.L.; WARD, D.C.; 'Isolation and initial characterization of a large repeat sequence element specific to mouse chromosome 8' * figure 8 * ---	1-12 -/-			
The present search report has been drawn up for all claims					
Place of search	Date of compilation of the search	Examiner			
THE HAGUE	15 April 1994	Nauche, S			
CATEGORY OF CITED DOCUMENTS					
X : particularly relevant if taken alone	T : theory or principle underlying the invention				
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